

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN No. 37.

B. T. GALLOWAY, *Chief of Bureau.*

FORMATION OF THE SPORES IN THE SPORANGIA OF RHIZOPUS
NIGRICANS AND OF PHYCOMYCES NITENS.

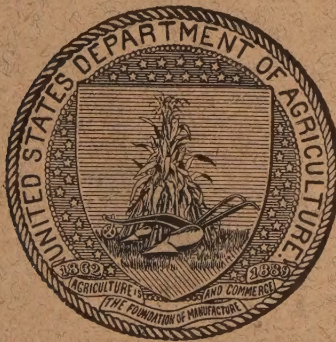
BY

DEANE B. SWINGLE,

ASSISTANT IN PATHOLOGY, LABORATORY OF PLANT PATHOLOGY.

VEGETABLE PHYSIOLOGICAL AND PATHOLOGICAL
INVESTIGATIONS.

ISSUED JUNE 27, 1903.



WASHINGTON.

GOVERNMENT PRINTING OFFICE.

1903.



BULLETINS OF THE BUREAU OF PLANT INDUSTRY.

The Bureau of Plant Industry, which was organized July 1, 1901, includes Vegetable Pathological and Physiological Investigations, Botanical Investigations and Experiments, Grass and Forage Plant Investigations, Pomological Investigations, and Experimental Gardens and Grounds, all of which were formerly separate Divisions, and also Seed and Plant Introduction and Distribution, the Arlington Experimental Farm, Tea Culture Investigations and Domestic Sugar Investigations.

Beginning with the date of organization of the Bureau, the several series of bulletins of the various Divisions were discontinued, and all are now published as one series of the Bureau. A list of the bulletins issued in the present series follows.

Attention is directed to the fact that "the serial, scientific and technical publications of the United States Department of Agriculture are not for general distribution. All copies not required for official use are by law turned over to the Superintendent of Documents, who is empowered to sell them at cost." All applications for such publications should, therefore, be made to the Superintendent of Documents, Union Building, Washington, D. C.

No. 1. The Relation of Lime and Magnesia to Plant Growth. I. Liming of Soils from a Physiological Standpoint. II. Experimental Study of the Relation of Lime and Magnesia to Plant Growth. 1901. Price, 10 cents.

No. 2. Spermatogenesis and Fecundation of *Zamia*. 1901. Price, 20 cents.

No. 3. Macaroni Wheats. 1901. Price, 20 cents.

No. 4. Range Improvement in Arizona. (Cooperative Experiments with the Arizona Experiment Station.) 1902. Price, 10 cents.

No. 5. Seeds and Plants Imported Through the Section of Seed and Plant Introduction for Distribution in Cooperation with the Agricultural Experiment Stations. Inventory No. 9, Numbers 4351-5500. 1902. Price, 10 cents.

No. 6. A List of American Varieties of Peppers. 1902. Price, 10 cents.

No. 7. The Algerian Durum Wheats: A Classified List, with Descriptions. 1902. Price, 15 cents.

No. 8. A Collection of Economic and Other Fungi Prepared for Distribution. 1902. Price, 10 cents.

No. 9. The North American Species of *Spartina*. 1902. Price, 10 cents.

No. 10. Records of Seed Distribution and Cooperative Experiments with Grasses and Forage Plants. 1902. Price, 10 cents.

No. 11. Johnson Grass: Report of Investigations Made During the Season of 1901. 1902. Price, 10 cents.

No. 12. Stock Ranges of Northwestern California. Notes on the Grasses and Forage Plants and Range Conditions. 1902. Price, 15 cents.

No. 13. Experiments in Range Improvements in Central Texas. 1902. Price, 10 cents.

No. 14. The Decay of Timber and Methods of Preventing It. 1902. Price, 55 cents.

No. 15. Forage Conditions on the Northern Border of the Great Basin, Being a Report upon Investigations Made During July and August, 1901, in the Region Between Winnemucca, Nevada, and Ontario, Oregon. 1902. Price, 15 cents.

No. 16. A Preliminary Study of the Germination of the Spores of *Agaricus Campestris* and Other Basidiomycetous Fungi. 1902. Price, 10 cents.

No. 17. Some Diseases of the Cowpea: I. The Wilt Disease of the Cowpea and Its Control. II. A Cowpea Resistant to Root Knot (*Heterodera radicicola*). 1902. Price, 10 cents.

No. 18. Observations on the Mosaic Disease of Tobacco. 1902. Price, 15 cents.

No. 19. Kentucky Bluegrass Seed: Harvesting, Curing, and Cleaning. 1902. Price, 10 cents.

No. 20. Manufacture of Semolina and Macaroni. 1902. Price, 15 cents.

No. 21. List of American Varieties of Vegetables for the Years 1901 and 1902. 1903. Price, 35 cents.

[Continued on page 3 of cover.]

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN No. 37.

B. T. GALLOWAY, *Chief of Bureau.*

FORMATION OF THE SPORES IN THE SPORANGIA OF RHIZOPUS
NIGRICANS AND OF PHYCOMYCES NITENS.

BY

DEANE B. SWINGLE,

ASSISTANT IN PATHOLOGY, LABORATORY OF PLANT PATHOLOGY.

VEGETABLE PHYSIOLOGICAL AND PATHOLOGICAL
INVESTIGATIONS.

ISSUED JUNE 27, 1903.



WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1903.

BUREAU OF PLANT INDUSTRY.

B. T. GALLOWAY, *Chief.*

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

SCIENTIFIC STAFF.

ALBERT F. WOODS, *Pathologist and Physiologist.*

- ERWIN F. SMITH, *Pathologist in Charge of Laboratory of Plant Pathology.*
GEORGE T. MOORE, *Physiologist in Charge of Laboratory of Plant Physiology.*
HERBERT J. WEBBER, *Physiologist in Charge of Laboratory of Plant Breeding.*
NEWTON B. PIERCE, *Pathologist in Charge of Pacific Coast Laboratory.*
HERMANN VON SCHRENK, *Special Agent in Charge of Mississippi Valley Laboratory.*
P. H. ROLFS, *Pathologist in Charge of Sub-Tropical Laboratory.*
M. B. WAITE, *Pathologist in Charge of Investigations of Diseases of Orchard Fruits.*
MARK A. CARLETON, *Cerealist in Charge of Cereal Investigations.*
WALTER T. SWINGLE, *Physiologist in Charge of Life History Investigations.*
C. O. TOWNSEND, *Pathologist.*
P. H. DORSETT, *Pathologist.*
T. H. KEARNEY, *Physiologist, Plant Breeding.*
CORNELIUS L. SHEAR, *Assistant Pathologist.*
WILLIAM A. ORTON, *Assistant Pathologist.*
FLORA W. PATTERSON, *Mycologist.*
JOSEPH S. CHAMBERLAIN, *Expert in Physiological Chemistry.*
R. E. B. MCKENNEY, *Expert.*
CHARLES P. HARTLEY, *Assistant in Physiology, Plant Breeding.*
DEANE B. SWINGLE, *Assistant in Pathology.*
JAMES B. RORER, *Assistant in Pathology.*
LLOYD S. TENNY, *Assistant in Pathology.*
JESSE B. NORTON, *Assistant in Physiology, Plant Breeding.*
A. W. EDSON, *Scientific Assistant, Plant Breeding.*
KARL F. KELLERMAN, *Assistant in Physiology.*
GEORGE G. HEDGCOCK, *Assistant in Pathology.*

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., February 20, 1903.

SIR: I have the honor to transmit herewith a technical paper entitled "Formation of the Spores in the Sporangia of *Rhizopus Nigricans* and of *Phycomyces Nitens*," and respectfully recommend that it be published as Bulletin No. 37 of the series of this Bureau. This paper was prepared by Mr. Deane B. Swingle, of the Pathological Laboratory of Vegetable Pathological and Physiological Investigations, and was submitted with a view to publication by the Pathologist and Physiologist.

Respectfully,

B. T. GALLOWAY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.



Digitized by the Internet Archive
in 2025

PREFACE.

The following paper by Mr. Deane B. Swingle, entitled "Formation of the Spores in the Sporangia of *Rhizopus Nigricans* and of *Phycomyces Nitens*," throws a new light on certain intricate processes in two important genera of fungi. The question of spore formation is one of vital interest to the study of the reproduction and distribution of fungi, both parasitic and nonparasitic. Mr. Swingle's paper corrects an erroneous idea that has received wide acceptance both in this country and abroad. The inherent properties and behavior of protoplasm must be the basis of work in pathology and physiology. This paper is a contribution to our knowledge, especially in regard to the mechanics of this type of cell-division, and to the nature and functions of the vacuole and the relation of the activities of the nucleus to those of the rest of the protoplasm. The results of this study are in a large measure applicable to many of the other fungi, including a number that are parasitic.

The paper is technical and is intended for the use of investigators in pathology and physiology.

ALBERT F. WOODS,
Pathologist and Physiologist.

OFFICE OF THE PATHOLOGIST AND PHYSIOLOGIST,
Washington, D. C., February 7, 1903.

CONTENTS.

	Page.
Historical.....	9
Methods.....	14
<i>Rhizopus nigricans</i> Ehrbg.....	15
<i>Phycomyces nitens</i> Kunze.....	23
General considerations.....	28
Summary.....	36
Index to literature.....	38
Description of plates.....	39

ILLUSTRATIONS.

	Page.
Plate I. <i>Rhizopus nigricans</i> . Fig. 1.—Group of sporangiophores with sporangia. Fig. 2.—Longitudinal section of young stolon. Fig. 3.—Longitudinal section of old stolon. Fig. 4.—Disintegrating nuclei from old stolon. Fig. 5.—Longitudinal section of young sporangium. Fig. 6.—Longitudinal section of nearly full-sized sporangium	40
II. <i>Rhizopus nigricans</i> . Fig. 7.—Longitudinal section of full-sized sporangium before the columella is formed. Fig. 8.—Longitudinal section of sporangium in which the columella is being formed. Fig. 9.—Section of small part of sporangium showing cleavage furrows..	40
III. <i>Rhizopus nigricans</i> . Fig. 10.—Longitudinal section of sporangium showing spore formation. Fig. 11.—Nuclei from columella that has just been formed. Fig. 12.—Sporangium in which the spores are completely formed. Fig. 13.—Nuclei from columella of old sporangium. Fig. 14.—Ripe spores in their living condition	40
IV. <i>Phycomyces nitens</i> . Fig. 15.—Longitudinal section of young sporangium. Fig. 16.—Small part of young sporangium very highly magnified. Fig. 17.—Formation of zones in sporangium. Fig. 18.—Layer of vacuoles in sporangium	
V. <i>Phycomyces nitens</i> . Fig. 19.—Formation of columella. Fig. 20.—Structure of vacuoles and nuclei. Fig. 21.—Formation of the spores. Fig. 22.—Furrows cutting outward from columella cleft. Fig. 23.—Furrows from the vacuoles cutting out to the periphery. Fig. 24.—Section showing nearly ripe spores. Fig. 25.—Ripe spores in their living condition. Fig. 26.—Peculiar-shaped spores. Fig. 27.—Very irregular-shaped spore	40
VI. Figures illustrating mechanics of cleavage. Fig. 28.— <i>Pilobolus</i> before formation of columella. Fig. 29.— <i>Pilobolus</i> during formation of columella. Fig. 30.— <i>Pilobolus</i> after formation of columella. Fig. 31.— <i>Pilobolus</i> during formation of spores. Fig. 32.— <i>Synchytrium</i> during formation of spores. Fig. 33.— <i>Fuligo</i> during formation of spores. Fig. 34.—Egg of squid during segmentation	40

FORMATION OF THE SPORES IN THE SPORANGIA OF RHIZOPUS NIGRICANS AND OF PHYCOMYCES NITENS.

HISTORICAL.

Although the life history and gross anatomy of nearly all the species of the Mucorineæ have been carefully worked over and described, yet in regard to the cytological details there are the widest differences of opinion, chiefly owing to the fact that only a few forms have been studied with the aid of the most recent methods. It seems desirable, therefore, that others should be critically examined. The present paper is a contribution toward that end.

The earliest account that deals specifically with the formation of the spores in the Mucorineæ is that of Corda (1838). He investigated the development of the sporangia of *Rhizopus nigricans*, but was able to discover little of the real nature of the process. After the formation of the columella in the lower part of the sporangium, he describes the spores as being formed in rows radiating from the columella, but just how they originate he does not make clear.

Van Tieghem (1873, 1875, 1876) in a series of classic papers has covered practically the entire group, describing the structure and development of a very large number of forms with much accuracy and minuteness of detail. He believed that the method of spore formation was the same in all the genera having a spherical sporangium. In these forms the sporogenous protoplasm separates itself into two very different substances—the sporal protoplasm which is always granular, and the intersporal protoplasm which is homogeneous and brilliant. The sporal protoplasm has the form of small polyhedric portions, and these are separated from each other by the intersporal protoplasm. Soon the polyhedric masses round themselves off, secrete a cellulose wall, and acquire the homogeneous refringent appearance which characterizes the spores of the greater number of the Mucorineæ. At the same time the intersporal protoplasm distributes itself so that it occupies all the space between the spores, and forms a layer between the peripheral spores and the sporangium wall. Van Tieghem considers this a process of free formation similar to that which occurs in the ascus, differing chiefly in the amount of intersporal protoplasm.

Strasburger (1880) has given an account of the more general features of the spore formation in *Mucor mucedo*. He considers that the sporogenous protoplasmic mass is cut up by cell plates analogous to those formed in cell division in the higher plants. This account is, however, very brief and incomplete.

Shortly afterwards, Büsgen (1882) studied the formation of the spores in *Mucor*. His conclusion is that the protoplasmic mass is cut up into blocks by cell plates, and that these blocks are subdivided until the final spores are reached. In this he adds little to Strasburger's account.

Léger (1896) published a paper intended to fill the gaps in our knowledge of the spore formation in the Mucorineæ. This paper is quite comprehensive, dealing with nearly all the principal genera. Léger studied the spore formation partly by means of sections of material embedded in collodion, but largely by examining the sporangia in toto or by crushing them under a cover glass. His results agree entirely with those of Van Tieghem. He finds that all the forms investigated agree in having the protoplasm divided at once into granular portions separated by nongranular plates. Later, the granular masses are surrounded by walls and become the spores, while the nongranular plates form the intersporal protoplasm.

In the case of *Rhizopus nigricans*, Léger finds that when the spores are first formed they are separated by thin membranes only. How these membranes originate he does not make clear. The intersporal substance appears a little later after the spore walls are formed. In this respect *Rhizopus* differs from all the other forms investigated.

In his description of the formation of the columella, Léger states that the contents of the sporangium are easily seen to be differentiated into a lighter and a denser portion. These are then separated by a columella wall, the lighter part being included in the columella and all the denser part remaining outside. Just how the protoplasm is divided and the wall formed he does not tell us. He states that the nuclei in the spores are oval, while those in the columella are spherical. As the spores ripen the cytoplasm disappears from the columella and the nuclei, reduced to nucleoli [sic], remain adhering to the inner surface of the columella wall.

The nucleus is essentially the same in all the forms which Léger describes. It consists of a nucleolus surrounded by a clear zone which does not stain, and outside of this by a distinct nuclear membrane. The nucleoli are described as so many times larger relatively than I have found them that I am entirely unable to credit his results. The nuclei, also, as he figures them, are much too large and contain no chromatin.

Thaxter (1897) has done the most to clear up our knowledge of the spore formation in the Syncephalidæ. He states that he was earlier

inclined to accept the view of Fischer (1892), which is that in such forms as *Syncephalis* the spores borne in a single row are formed exogenously by constriction like conidia, the wall of the fruiting body forming part of the spore wall, and that this body can not, therefore, be considered as a sporangium homologous with that of *Mucor*. After a thorough study of *Syncephalastrum* and *Syncephalis*, however, he accepted the "sporangial" theory, and brings very conclusive evidence to support his results. In *Syncephalastrum racemosum* he finds that the contents of the cylindrical cells that are to form the chains of spores are divided into spores, not by gradual constriction from the surface inward, but simultaneously by a hyaline intersporal substance. Walls are then formed around the individual spores entirely within and distinct from the wall of the mother cell. By crushing these spore rows under a cover glass he was able to force the spores out in a perfect condition, leaving the walls of the sporangia empty and intact except for their ruptured tips. This is conclusive evidence of the endogenous formation of these spores. Furthermore, in many cases he finds that the spores are borne, not in single rows, but more or less irregularly, the diameter of the sporangium being somewhat greater than that of a single spore. In such cases the planes of separation are oblique, or even parallel, to the long axis of the sporangium. In such a form as this Thaxter finds an intermediate stage between the spherical sporangium of *Mucor* and the cylindrical one of *Syncephalis*, the supposed absence of which was used by Fischer as evidence against the homology of the two.

In *Syncephalis*, Thaxter finds that the separation of the protoplasm into spores is quite different from that in *Syncephalastrum*. He investigated an undescribed species from Liberia, and also *Syncephalis pycnosperma*, and finds that in both cases the protoplasm is cut progressively from the surface inward by "intermediary zones," each of which is made up of an inner nonstainable part, and an outer one that takes stains readily. The spore wall in both species is distinct from the sporangium wall and forms close around the protoplasm, excluding the intermediary zones. In the undescribed species these zones remain until the spores are ripe and then deliquesce, while in *Syncephalis pycnosperma* the stainable portion breaks up into a refractive oily substance and the nonstainable part forms a thick permanent layer around the spore wall and gives to the spores their peculiar shape.

Harper (1899) has described the spore formation in *Pilobolus* and *Sporodinia* of the Mucorineæ, and also in *Synchitrium* of the Chytridiaceæ. The processes in these widely separated forms show many interesting points of similarity.

In *Synchitrium*, Harper finds that the "initial cell" contains at first one comparatively large nucleus, which, as the cell reaches nearly its

full size, divides rapidly to form a vast number of smaller daughter nuclei. This multinucleated mass of protoplasm is then divided into comparatively large blocks by narrow furrows, cutting progressively inward from the periphery. These furrows cut inward at nearly right angles to the periphery, but, as seen in surface sections, they intersect each other at almost every angle. They are so narrow that they appear in section as single lines which push aside the vacuoles, arranging them in a row on either side. In case the sporangium is slightly shrunken in fixing, however, they appear as slightly separated surfaces. As these cleavage furrows grow deeper they branch, curve, and intersect each other until the whole mass is divided into multinucleated pieces. These are then divided into uninucleated pieces by furrows cutting inward from their surfaces.

The nuclei then divide until there are usually from 8 to 12 in each piece. Without further cleavage these multinucleated protoplasmic masses then enlarge somewhat, secrete a protective wall, and become the spores. They then go into a resting condition until germination.

In *Pilobolus*, Harper traces the entire development of the sporangium. He finds that when it has reached a considerable size its contents are divided into three parts—a central vesicle of cell sap, which, from the absence of a smooth, rounded surface, can not be considered as a central vacuole; outside this, a thin layer of spongy protoplasm with numerous nuclei; and outside this layer, extending to the sporangium wall, a much denser mass of protoplasm, also containing many nuclei and a few rounded vacuoles. In the spongy protoplasm, and running parallel to the sporangium wall except at the lower side where it extends to the periphery, a dome-shaped layer of vacuoles then appears. These vacuoles are at first round, but later they become flattened parallel to the surface of the sporangium until they are disk-shaped. They finally fuse, edge to edge, to form a cleft, which, with the aid of a circular furrow cutting upward through the spongy protoplasm until it meets the lowest vacuoles in the series, cuts out the columella. This columella is bounded at first by only a plasma-membrane, outside of which is a more or less open cleft. Later the columella wall is formed in this cleft. It has its dome-shaped outline from the first, and does not begin as a cross wall at the base of the sporangium, being rounded upward later by pressure of turgor from below, as is described for *Mucor* in most standard text-books. (See Bessey's text-book, p. 236.)

The spore plasm is then invaded by surface furrows cutting progressively inward. These are much like those in *Synchytrium*, but wider, owing to the more shrunken condition of the protoplasm during the process. While this is going on, the vacuoles in the spore plasm become sharply angular, and these angles, continuing outward as furrows, cut into each other and into the furrows from the surface,

thus aiding in the cleavage. The whole mass is thereby reduced to blocks of varying sizes which are, as in *Synchitrium*, progressively cut down to uninucleated pieces. As in *Synchitrium* also, these protospores are pressed tightly together by turgor.

The nuclei then divide until there is a considerable number in each piece of protoplasm. This division is followed by successive constrictions of the nature of bipartitions until a binucleated stage is reached. Each piece then surrounds itself with a wall and is a mature spore. The later phases of the process—i. e., from the protospore to the mature spore—Harper regards as an embryonic development.

In the subdivisions of the protosporés, Harper notes that the protoplasm in advance of the cleavage furrows becomes clear and non-stainable, forming a hyaline zone in the plane of constriction, as though the denser part of the protoplasm drew away from this region toward the nuclei, leaving only a clear liquid substance behind. In the earlier stages of cleavage, however, both in *Pilobolus* and in *Synchitrium*, such a differentiation of protoplasm in advance of the cleavage furrows does not take place.

Here, as in *Synchitrium*, the entire protoplasm is included in the spore, there being no intersporal protoplasm. There is a slime excreted to fill the spaces between the spores, but it is not protoplasm.

In *Sporodinia* the process is in many respects much like that in *Pilobolus*, but there are some striking differences. The sporangia here are much smaller and are composed of two parts, the outer and upper part being filled with dense protoplasm, while the central and lower portion is occupied by a foamy protoplasm, there being no large opening filled with cell sap as in *Pilobolus*. The vacuoles that cut out the columella are much larger than in *Pilobolus*, and are arranged on the line, as it appears in section, between the two kinds of protoplasm. They fuse laterally to form a curved cleft, but no surface furrow cutting in to meet them has been observed. The spore plasm is then divided into blocks by furrows cutting from the columella cleft outward and from the surface inward, but here the cleavage process ceases. No uninucleated stage is ever reached. These protoplasmic blocks contain numerous nuclei, and round off and are covered with a cell wall. They are then the mature spores. This is a considerable abbreviation of the process in *Pilobolus*, and there is a corresponding shortening in the time required for developing the spores in *Sporodinia*.

The nuclei in all three forms are made up of the same parts as those in the higher plants. There is a nucleolus surrounded by a zone filled with nuclear sap and chromatin, the whole being enveloped in a nuclear membrane. A point well worthy of consideration is that the nuclei are in a resting condition during cleavage.

Hans Bachmann (1899) has described the entire structure and

14. FORMATION OF SPORES OF RHIZOPUS AND PHYCOMYCES.

development of a new species of *Mortierella*. Though the paper was published very recently, little improvement over the older writers is shown in the matter of technique. He has not, so far as he states, made any sections of the sporangia.

By a study of entire sporangia he finds that the surface comes to be marked out into polygons separated by rather broad bands of an even width. These markings he interprets as representing a surface view of polyhedral masses of protoplasm which are destined to become spores, separated by layers of intersporal protoplasm.

Plasmolyzing agents in some cases cause the sporangium to contract as a unit and not as individual polygons, showing that each is not yet entirely surrounded by an osmotic membrane.

Gentian violet stains the material between the polyhedrons; the formation of the violet lines is progressive, as is shown by the fact that in some cases they are short and do not extend over the entire sporangium, but radiate from various points. In this, Bachmann makes a decided advance over Léger, but still he apparently fails to grasp the most important point—that these blue-staining lines represent cleavage furrows filled with the stain.

METHODS.

The mold *Rhizopus* was first obtained in mixed cultures by exposing moistened bread for a few minutes to the air of the laboratory. To obtain pure cultures, a few sporangia were carefully transferred from the original cultures to slightly moistened bread, which had been exposed an hour or so on two or more successive days to a temperature of from 60° to 65° C. in a steam sterilizer. In from one to two days after inoculation the stolons began to appear on the surface of the bread, and in another day there were a considerable number of sporangia formed.

The cultures of *Phycomyces* were obtained from Ann Arbor, Mich., through the kindness of Dr. J. B. Pollock. This mold was grown either upon sterilized bread or nutrient agar. From these cultures small bits of mycelium were cut out (below the surface of the substratum in the case of *Phycomyces*) and instantly immersed in the fixing fluid. After remaining in this about twenty-four hours, they were washed for a few hours in running water, dehydrated by running through grades of alcohol, cleared in xylol or chloroform, and embedded in paraffin.

The sections were cut on a Jung, or a Reinhold-Giltay microtome, usually 4 μ thick, but sometimes 2 μ , and were fastened to slides with albumen and glycerine. They were then stained with Flemming's triple stain (safranin, gentian violet, and orange G), then dehydrated, cleared with clove oil or bergamot oil, and mounted in Canada balsam. If the right exposures are given to these stains, the cytoplasm

appears orange, the chromatin blue, the nucleolus and proteid crystalloids red, and the cell wall either blue or orange.

For fixing fluids the mixtures of Flemming, Hermann, and Merkel were used with very good results. Eisen's fluid gave some very fine results, but was little used. An exposure of one hour to Flemming's fluid, followed by twelve to twenty-four hours in Merkel's fluid or chrom-acetic acid, gave especially fine preparations, not being so much blackened as when exposed longer to the osmic acid.

I am deeply indebted to Dr. Robert A. Harper of the University of Wisconsin, and to Dr. Erwin F. Smith, Dr. Rodney H. True, and Mr. Karl F. Kellerman of the United States Department of Agriculture for many valuable suggestions and criticisms given during the progress of the work.

RHIZOPUS NIGRICANS Ehrbg.

The general morphology of *Rhizopus* has been very well described by the earlier authors.

The spore in germinating sends out a tube which branches until a tangled mycelium is formed in the substratum. This mycelium sends up from various points aerial hyphæ, which are erect at first and form a delicate white growth in the cultures. After these hyphæ reach the height of one or two centimeters they bend over and grow horizontally along the surface of the substratum.

When one of these stolons has grown in this direction for a short distance, it forms a swelling at the apex two to four times the diameter of the stolon, and out of this grow from two to six branches, one of which is in reality a continuation of the stolon, while the others grow into sporangiophores. (Pl. I, fig. 1.) If this swollen portion of the stolon comes in contact with the substratum or the sides of the culture dish, a few rhizoids are sent out which firmly anchor it, and, in case they penetrate any nutritive substance, these doubtless aid in nourishing the sporangiophores. The stolon continues growing out and forming these groups of sporangiophores at intervals, and finally ends with such a group at the apex. Each sporangiophore bears a single spherical sporangium.

In healthy stolons, especially if they are growing rapidly, the protoplasm is almost continually streaming in one direction or the other. This has been fully described by Arthur (1897), who considers that it is principally due to evaporation of moisture from the surface of exposed parts, together with the constant taking in of water by the hyphæ that are in the substratum. In his conclusion he expresses the opinion that "the movement is an incidental feature in the life of the plant." Further mention of this paper will be made in connection with the distribution of the protoplasm in the sporangium.

The growing ends of the stolons are densely crowded with protoplasm containing many nuclei. This condition prevails for some distance back in the stolons (Pl. I, fig. 2), but as we follow back toward the older part the protoplasm is more and more permeated with cell sap, and at last we find a region where there is nothing but a wall filled with cell sap, so far as we can distinguish from a surface view of living material. In stained sections, however, as shown in Pl. I, fig. 3, it can be seen that there is still a thin layer of protoplasm lining the wall, and strands or even small masses of it in the center. In parts as old as that shown in the figure, the nuclei have begun to disintegrate somewhat, and appear as tiny red-staining masses of various shapes. (Pl. I, fig. 4.)

The young sporangiophores, like the ends of the stolons, are densely crowded with protoplasm and nuclei, and even the lower part of the older ones is never entirely devoid of protoplasmic contents, as is stated by Léger, but retains a structure very much like that in the stolons.

As the sporangiophore reaches its full length it begins to swell out at the tip into a tiny round body, the future sporangium. The contents of this are at first evenly distributed, being equally dense in the center and at the periphery, but before it has reached half its final size the protoplasm begins to be decidedly dense toward the sporangium wall, while in the center it is of a much looser structure. Pl. I, fig. 5, shows the distribution of the cytoplasm and nuclei at this stage. There are also present a few crystalloids. They seem often to be in tiny clear vesicles, but whether or not these are ordinary vacuoles I can not be certain. These crystal-like bodies vary much in size, and as a rule increase in number as the sporangium gets older. It is quite noticeable, however, that they are entirely confined to the central part of the sporangium.

The nuclei are so small that they appear only as dots in a drawing of the size of Pl. I, fig. 5. Their structure can, however, be clearly made out with higher magnification, and it is to all appearances precisely like that of those shown in Pl. II, fig. 9, which will be described later.

The cytoplasm in young sporangia, it will be observed, is quite dense next the sporangium wall, but gradually becomes less dense toward the center, where it is of a very loose spongy structure, containing many vacuoles of considerable size. There is at this stage no sharply defined boundary between the denser and the less dense parts of the cytoplasm, but a gradual transition from center to periphery. The denser layer does not, however, extend quite to the sporangiophore at the base of the sporangium. (Pl. I, figs. 5 and 6.)

At this time also there is a very marked streaming of the protoplasm up the sporangiophore into the sporangium. These currents appear

as a bundle of strands, which in optical section spread fan-like as they enter the sporangium and extend toward the periphery. Many of these streams, particularly at the sides, extend nearly to the sporangium wall, as seen in Pl. I, fig. 5. Harper (1899) has described each individual current in *Pilobolus* as having "marked a path for itself through the protoplasmic structure. It is marked by continuous delicate films, quite distinct from the spongy structure of the adjacent plasma." These surrounding films, as the writer has seen them in *Rhizopus*, are of a more hyaline and homogeneous appearance than either the currents or the surrounding cytoplasm.

The nuclei in these currents are much elongated in the direction in which the currents run and I have not been able to differentiate the parts, the nucleolus and chromatin both staining red.

As the streaming continues the protoplasm at the periphery becomes denser, and there appear clearly differentiated layers within the sporangium. The beginning of this differentiation is not simultaneous throughout the protoplasm. It appears at certain points approximately equidistant from the periphery, between which and the periphery the thickening of the protoplasm forms a dense zone lining the sporangium except at the base, where its inner boundary line gradually extends to the periphery. In the stage shown in Pl. I, fig. 6, this boundary line is not perfect, but somewhat broken, admitting thin streams of loose protoplasm from the interior of the sporangium. Inside this zone and of about one-third its thickness is a semitransparent layer consisting of loose protoplasm like that which fills the interior of the sporangium, but clearer and less granular, and taking the orange stain less strongly than the latter. In structure it resembles the thin films about the streams previously mentioned. The cytoplasm inside this semitransparent zone and occupying the central and lower part of the sporangium is of a loose, spongy, much-vacuolated structure, containing scattering nuclei and a considerable number of proteid bodies. There are no marked protoplasmic strands indicating currents in the center of the sporangium, though the writer has often found them in later stages; but radiating from the central part of the sporangium and passing from it through the clear zone to the denser plasma are many very slender strands marking the paths of currents. These currents bear nuclei and seem to represent a very late stage of the migration of cytoplasm and nuclei toward the periphery. Some of these streams enter the openings in the denser plasma, while others run against its inner surface. This streaming goes on until the inner boundary of the denser plasma is at all points sharply defined. This boundary does not consist of a membrane or of any differentiated layer.

The denser plasma at this time contains only a very few vacuoles of any considerable size, but under very high magnification it can be seen

that there are very many exceedingly small ones, with definitely rounded outlines. Most of these are scarcely larger than the nuclei, and some are much smaller. They can not, therefore, be shown in a drawing on so small a scale as Pl. I, fig. 6. They are, however, essentially the same in size, number, and distribution as those shown in Pl. II, fig. 9.

Thus far, except for the arrangement of the cytoplasm and nuclei, we have had no phenomena in the sporangium that even suggest cell division, unless possibly it be the clear zone. The greater part of the more solid portion of the cytoplasm has formed itself into a layer at the periphery. Nearly all of the nuclei also have migrated into this portion of the sporangium, and are distributed irregularly throughout the dense cytoplasm. They are not even approximately equidistant from each other, nor are they often, if ever, in actual contact, though Léger states that such is very frequently the case. How he could determine the normal distribution of the nuclei from crushed sporangia is difficult to comprehend.

As soon as the protoplasm is distributed as has been described, the separation of that which is to be included within the columella from that which is to form the spores begins. The columella is not at first a flat cross wall at the base of the sporangium which is later pushed up by turgor to its characteristic dome shape, as it is currently described as doing, but is laid down in essentially the same fashion as described by Harper (1899) for *Pilobolus*. There first appears in the denser plasm a single layer of spherical vacuoles (Pl. II, fig. 7) running parallel to its inner surface. The layer of the denser plasm inside the system of vacuoles is usually from one-fifteenth to one-twentieth as thick as the layer outside. Apparently these vacuoles are formed by the enlargement of the very minute ones already mentioned that lie in this region, rather than by the migration of previously enlarged vacuoles. In sporangia in which this layer of vacuoles is only partly formed there are usually a few large vacuoles arranged in the layer, and between them are smaller ones, varying in size down to the smallest in the sporangium (Pl. II, fig. 7). This leads one to believe that the vacuoles in this layer are essentially like the others in the sporangium and in the mycelium. These vacuoles and all others in the sporangium agree with those of *Pilobolus* and *Sporodinia* in being devoid of all stainable contents (Pl. II, fig. 7), in which respect they differ strikingly from those of *Phycomyces*, described later.

The vacuoles are at first spherical, or nearly so, but soon begin to flatten, their long axes being parallel to the inner surface of the denser plasm. By this flattening they become disk-shaped, as in Pl. II, fig. 8, and the edges of adjacent ones come in contact and fuse, forming a narrow curved cleft in the protoplasm. At the same time a circular furrow begins to cut upward from the surface of the protoplasm at

the base of the sporangium through the denser plasm (Pl. II, fig. 8). This furrow increases in depth until it reaches and fuses with the lowest vacuoles in the layer. Thus the protoplasm of the sporangium is divided into two distinct portions destined to perform radically different parts in the further life of the plant. That outside the cleft is to be entirely cut up into spores, while that inside is later to be surrounded by the columella wall and plays no direct part in reproduction. The former I shall distinguish as the spore-plasm and the latter as the columella-plasm. It will be noted from what has been already said and from Pl. II, figs. 7 and 8, and Pl. III, figs. 10 and 12, that the columella-plasm includes all the looser plasm in the sporangium and also a thin layer of the denser plasm.

One might have expected from Pl. I, fig. 6, that the columella wall would be laid down in the clear zone shown in that figure, but that such is not the case there is no room for doubt. The writer has preparations in which this zone is still almost as marked as in the figure mentioned, while the columella cleft is forming in the denser plasm. Pl. II, fig. 8, and Pl. III, fig. 10, show that the outer part of the looser plasm is still somewhat clearer than that in the center, though the paths of the currents have become almost obliterated. The time for the disappearance of the currents varies greatly in different sporangia.

There is no visible difference while cleavage is going on between the denser plasm inside the layer of vacuoles and that outside, nor is there any differentiation of the cytoplasm between the vacuoles or in advance of the surface furrow, such as Harper found in the late subdivisions of the protoplasm of *Pilobolus* and in the last stages of cleavage of *Fuligo* (1900).

While the cutting out of the columella is going on, the sporangium gives every appearance of having only slight turgidity. The cleft in the protoplasm is always quite wide—at least in certain places. When, however, the cleavage is complete, the protoplasmic masses increase in volume and become strongly turgid again, causing the two protoplasmic surfaces lately separated to become pressed together so tightly that only by the closest study can one follow the cleft throughout its entire extent.

In case the spore cleavage, which will be described later, begins before the columella cleft is completed, as often occurs, this period of turgidity is postponed until after the spores are entirely cut out.

It will be noted that when first formed the cleft around the columella is bounded by two protoplasmic surfaces. When these surfaces become tightly pressed together by the turgor in the sporangium, one might expect them to fuse into a continuous mass of protoplasm again, there being no wall between them at this time. Indeed, such a phenomenon was described by Büsgen (1882) in the formation of the

spores of the *Saprolegniae*. It is not, however, surprising that with the technique used in those days he should fail to see that there was still a distinct boundary between the closely packed spores.

When the period of turgor relaxes a little the two surfaces generally separate slightly, but at irregular intervals points are often found where they still adhere, forming tiny conical projections, whose apices are for a short time in contact.

In the behavior of these two protoplasmic surfaces we have considerable additional evidence for the existence of a definite plasma-membrane.

Even before the cutting out of the columella takes place the nuclei of the looser protoplasm begin to disintegrate. In very young sporangia all the nuclei have the same normal structure, but in the one shown in Pl. I, fig. 6, for example, they are clearly suffering disintegration in the center of what is to become the columella-plasm, though out near the denser plasm they retain their characteristic structure, often until the spores are nearly ripe. (Pl. III, fig. 13, *a*.)

It might be suggested that the nuclei in the center of the sporangium are not well fixed, but these sporangia are so small and thin-walled that I can not believe, with all the cytoplasm and the greater part of the nuclei having a perfectly normal structure, that the difference in appearance of these nuclei is to be attributed to poor fixation, especially as it is essentially the same for all the best fixing fluids used.

The first sign of disintegration is the appearance of a red-staining mass on one side. As the process goes on, the whole nucleus comes to appear as a slightly shrunken, homogeneous mass, often irregular in shape, and staining the same shade of red as the crystalloids. It might be argued that these red-staining bodies are crystalloids whose substance is being dissolved, but I have found very good evidence that such is not the case. As shown in Pl. III, figs. 11 and 13, there are all stages of disintegration between the almost perfect nuclei and the most shrunken and angular ones. On the other hand, all the crystalloids in these sporangia, so far as could be observed, are perfect in shape, none showing notches or marks of corrosion, such as we should expect to find if they were being dissolved. Furthermore, the crystalloids seem to be forming rather than dissolving, judging from their greater number and size in the older sporangia.

In Pl. III, figs. 11 and 13, *a* represents a nucleus with normal structure lying just inward from the denser plasm, while *b*, *c*, and *d* lie nearer the center and are breaking down. In no sporangia as old as that shown in Pl. I, fig. 6, have I found nuclei in or near the center of the looser plasm in which nuclear membrane, chromatin, and nucleolus could be distinguished. These nuclei do not entirely disappear during the life of the plant, nor would it be at all accurate to say, as Léger has done, that they are "reduced to a nucleole."

The formation of the spores usually begins after the columella cleft is complete, although in some instances (as in Pl. II, fig. 8) somewhat previous to that, but always before the laying down of the columella wall. Spore formation does not take place in the manner described by Van Tieghem and Léger—by the simultaneous differentiation of plates of hyaline nongranular protoplasm cutting the spore-plasm into polyhedric blocks—nor by the progressive differentiation of such plates from lines on the surface of the protoplasm, as described by Bachmann (1900). In the scores of sporangia sectioned in all stages of development the writer has not found at any time even the slightest indication of such a differentiation of the protoplasm into granular polyhedric masses with nongranular plasm between. The first indication of the division of the spore-plasm is the formation of furrows at the surface, which cut progressively inward. (Pl. II, figs. 8 and 9.) These furrows are not broad, as in *Pilobolus*, nor are their sides closely pressed together, as in *Synchitrium*. They cut in at very different angles to the surface of the sporangium, and pass between, and often very close to, nuclei and vacuoles. (Pl. II, fig. 9.) They usually branch or curve at a short distance inward from the surface, and by cutting into and fusing with neighboring furrows cut out small pieces of the surface layer of the protoplasm of the sporangium. These pieces are almost always the definitive spores, lacking only the walls. Only a few of the larger ones are further divided up. There is no uninucleated stage in the spore formation of *Rhizopus*, as in *Pilobolus*, it being like *Sporodinia* and *Phycomyces* in this respect. These spores are at first somewhat angular in shape and contain exactly the same number of nuclei (2 to 6) as when ripe, there being no nuclear division at any stage of their existence previous to germination.

The nuclei of the spore-plasm during all stages of cleavage are in a resting condition. (Pl. II, fig. 9.) Each consists of a nucleolus, or occasionally two nucleoli, which in my preparations is stained a deep red, surrounded by a zone of evenly granular, blue-staining chromatin, the whole being bounded by a definite nuclear membrane. Both in the spore-plasm and in the columella the nuclei are spherical or very slightly ovoid until they begin to disintegrate. They are relatively more numerous in some sporangia than in others, which may possibly be due to differences in the moisture supply, wet cultures making looser and more bulky cytoplasm than drier ones.

The vacuoles of the spore-plasm, which are for the most part exceedingly minute, as can be seen by a comparison with the nuclei in Pl. II, fig. 9, do not become angular and assist in dividing the protoplasm here as in *Pilobolus* and *Phycomyces*. They retain their rounded form throughout the entire process of cleavage, even when furrows cut very close to them. As previously stated, they contain nothing but ordinary cell sap.

After the surface furrows have cut inward for a considerable distance, a few similar furrows begin to cut outward from the columella cleft, which as yet contains no wall. (Pl. III, fig. 10.) With the meeting of these two systems of furrows the cleavage is practically complete.

During the process of spore cleavage the protoplasm is slightly shrunk, apparently because of the giving off of water. The furrows are more or less open and filled with clear cell sap only. (Pl. II, fig. 9.) As soon, however, as the cleavage is complete, the spore mass becomes strongly turgid again, and each spore so increases in volume that all are pressed tightly together and the furrows are entirely closed, so that with the Zeiss 2 mm. immersion objective, 1.30 aperture, and No. 18 compensating ocular, they appear in optical section as single lines and are very hard to trace through the dense spongy cytoplasm. The spores are thus made sharply angular, but later they round off, leaving little spaces between them.

The formation of the columella wall usually begins before the spores are entirely cut out, but it does not reach its definitive thickness until they are nearly ripe.

As seen in Pl. III, fig. 12, these spores have no regular system of arrangement whatsoever, and the writer can not find the slightest ground for Corda's view that they are in radial rows.

As already stated, the spaces between the spores contain at first absolutely nothing except cell sap. There is no trace of any intersporal protoplasm, such as has been described by the earlier authors and considered as homologous with the epiplasm of the ascus.

The spores of *Rhizopus* are at first angular and covered by only a plasma-membrane, but soon round off and a firm wall is formed about them.

During this process of ripening a homogeneous slime is excreted by the spores, which fills up the spaces between them. In such exposures to the triple stain as best bring out the cytoplasmic and nuclear structures this intersporal slime does not stain at all, and for this reason the writer has left it an empty space in Pl. III, fig. 12. By a longer exposure to the violet it is readily brought out as a smooth bluish mass filling up the spaces between the rounded spores.

There is no special mechanism for the discharge of the spores in *Rhizopus* as in *Pilobolus*. There is, however, an inner layer of the sporangium wall that can not readily be differentiated from the rest of the wall in specimens fixed in the killing fluids of Flemming and Merkel; while in those fixed in Eisen's fluid and stained in the triple stain it is very readily distinguishable from the outer layer by its lighter blue color, the boundary between the two being sharply defined. Pl. II, fig. 7, is therefore the only one in the writer's series in which he could show the separate layers of the sporangium wall.

The inner layer is somewhat thicker than the outer, both being of an even thickness except for a little space around the sporangiophore where the inner one thins out and disappears. Whether or not this is homologous with the "collar" of *Pilobolus*, the writer can not be certain.

The spores are set free by the bursting of the sporangium wall, without its being thrown off. Whether or not the inner layer of the wall swells by the absorption of water and bursts the outer layer the writer has not determined. The writer has never found this inner layer on sporangia as young as that shown in Pl. I, fig. 5, nor in the walls of the mycelium.

The ripe spores as they escape from the ruptured sporangia are mostly ovoid in shape and of varying sizes. Their walls are marked with longitudinal ridges, as may be seen in Pl. III, fig. 14.

PHYCOMYCES NITENS Kunze.

Unlike *Rhizopus*, the sporangiophores of *Phycomyces* are borne singly, springing directly from the mycelium. When the sporangiophore is yet only a few millimeters long, the apex begins to swell out into a sporangium in the same manner as that described for *Rhizopus nigricans*. As the sporangium enlarges the sporangiophore elongates, pushing up the former farther and farther from the surface of the substratum. The spores are formed when the sporangiophore is about 2 cm. long, and it is then that the sporangium has its maximum diameter.

As shown in Pl. IV, fig. 15, there is the same streaming of cytoplasm and nuclei up the sporangiophore and out toward the periphery of the sporangium as in *Rhizopus nigricans*.

As can be seen by a comparison of Pl. IV, fig. 15, with Pl. I, fig. 5, the cytoplasm in the young sporangium of *Phycomyces* is more coarsely granular than that of *Rhizopus* and takes the stain much more deeply.

The most noticeable difference between the young sporangium of *Phycomyces* and that of *Rhizopus* is that in *Phycomyces* there are many more large round vacuoles which, as they move outward toward the periphery of the sporangium, become filled with a visible content. (Pl. IV, fig. 15.) This content appears in sections stained with the triple stain as a bluish homogeneous body of the same shape as the vacuole but somewhat smaller in diameter, lying in the middle of the vacuole, with a clear zone between it and the vacuolar membrane. (Pls. IV and V, figs. 15 to 22.) This content begins, not as a very minute, sharply-staining body which grows larger and larger in diameter, but as a faintly-staining mass which, as it grows older, becomes more dense and takes the stain more strongly. In the youngest stage it appears quite as large in proportion to the size of the vacuole in which it lies as when it becomes older. (Pl. IV, fig. 16.) It forms in

the vacuoles *after* they have entered the sporangium—never, so far as I have observed, appearing in those of the mycelium or sporangiophore, and rarely in those of that part of the sporangium which lies close to the mouth of the sporangiophore. The younger stages of their formation are shown in Pl. IV, figs. 15 and 16, while in Pls. IV and V, figs. 18, 19, and 20, they have reached their maximum density.

As the protoplasm streams up into the sporangium and out toward the periphery, there is at first a gradual transition in density from the center outward, precisely as in *Rhizopus* at the same stage. (Pl. IV, fig. 15.) A little later, however, as in Pl. IV, fig. 17, it is divided into three regions, differing in density. The outer region or layer is very dense and takes the stain strongly. Inside this is a second layer, which is considerably less dense and stains less strongly. Inside this second layer and occupying the central part of the sporangium is a region of very loose and much vacuolated protoplasm which takes the stain scarcely at all. Between the interior region and the second layer the differentiation becomes very sharp, but, as in *Rhizopus*, there is no wall or membrane of any kind between them. Between the second and the outer layers, however, the transition is at first very gradual (Pl. IV, fig. 17), but becomes more and more sharp as the sporangium grows older. I have never found in *Phycomyces* a stage such as is shown in Pl. I, fig. 6, which occurs regularly in *Rhizopus*. It is possible that this second layer is homologous with the semitransparent zone that has the same relative position in the sporangium of *Rhizopus*. I have not regarded it as such, however, as it is of so much greater relative density and contains no delicate strands representing currents. It is interesting in this connection to compare Pl. I, fig. 6, with Pl. IV, figs. 17 and 18.

The nuclei are at first about evenly distributed in the outer and second layers, but in the interior there are very few, or for a short period in the development of the sporangium none. (Pl. IV, figs. 17 and 18.)

None of the vacuoles in the interior region of very loose protoplasm or in the inner part of the second layer has the stainable content mentioned above. Practically all of the larger ones in the outer dense layer contain this substance, however, as also do most of those in the outer part of the second layer. (Pl. IV, fig. 17.) Between these larger vacuoles are very small ones which contain nothing that takes the stain. (Pls. IV and V, figs. 15–24.) The difference in the destinies of these two kinds of vacuoles will be seen later.

As may be seen from Pl. IV, figs. 15, 17, and 18, and Pl. V, fig. 19, the vacuoles that contain the stainable substance are very numerous, taking up a considerable portion of the space in the sporangium and lying very close together, often two or more being in actual contact, their clear zones being separated by only the vacuolar membranes. (Pls. IV and V, figs. 15, 17, 18, 19, and 20.) In such cases

the vacuolar membrane is isolated from the remainder of the protoplasm for a little space, and may readily be seen and studied by itself. (Pl. V, fig. 20.) It is very thin and homogeneous, taking the violet stain very slightly, which gives it a faint blue color. When two vacuoles are thus in contact they are usually flattened against each other, so that the membrane between appears in optical section as a thin, straight line. In such cases the contents are often flattened on that side to conform to the shape of the vacuole. (Pl. V, fig. 20.)

A considerable number of the nuclei that are in the second layer when it is first formed migrate into the denser plasm, and the differentiation between the two layers becomes more distinct. Then a layer of vacuoles, practically all having stainable contents, becomes arranged in a dome shape in the denser plasm and running parallel to its inner surface. (Pl. IV, fig. 18.) These vacuoles flatten out, become disk-shaped, and fuse edge to edge to form a dome-shaped cleft in the denser plasm, as in *Rhizopus* and *Pilobolus*. (Pl. V, fig. 19.) It is interesting to note that as the vacuoles flatten, the content flattens also, so that its surface remains always more or less parallel to the vacuolar membrane. (Pl. V, fig. 19.)

So far as I have been able to observe, there is never a surface furrow that cuts inward to meet the lowest of the layer of vacuoles, as is the case in *Pilobolus* and *Rhizopus*. In this respect *Phycomyces* appears more like *Sporodinia*. The layer of vacuoles begins so very near the surface of the protoplasm (Pl. V, fig. 19) that if there is such a surface furrow it must be very shallow indeed. I have never found any evidence of its existence.

When the vacuoles of this layer have entirely fused, edge to edge, the separation of the columella is complete. There is at first no wall—simply a cleft bounded by plasma-membranes. The contents of all the vacuoles that make this cleft have now fused, forming a layer of slightly uneven thickness separating the outer surface of the columella plasm from the inner surface of the spore-plasm. All the very loose interior protoplasm, the second layer, and a small part of the denser plasm are included within the columella, while the greater part of the denser plasm goes to form the spores.

As soon as the differentiation of the columella is complete, or in exceptional cases a little before, the formation of the spores begins. Here we get a most striking difference between *Phycomyces* and *Rhizopus*. The large round vacuoles in the spore plasm begin to lose their rounded form and become angular. (Pl. V, figs. 21 and 22.) These angles become sharper and sharper, and appear to cut through the cytoplasm between the nuclei, and when they encounter each other fuse to form irregular clefts. The cytoplasm in advance of these vacuolar furrows shows no visible differentiation, but remains of an even density throughout the entire spore-plasm during the whole process of

cleavage. (Pl. V, figs. 21 and 22.) So far as the writer has been able to observe after a most diligent search in a very large number of sporangia in all stages of spore formation, there are never surface furrows cutting into the spore-plasm at any point. The angles from the vacuoles may often be seen cutting out to the surface of the spore-plasm. (Pl. V, figs. 21 and 23.) Furrows also cut into the spore-plasm from the columella cleft and fuse with the vacuolar furrows in the spore-plasm, and thus aid in dividing the protoplasm into spores. (Pl. V, fig. 22.)

During the whole process of spore formation the nuclei are in a resting condition. They are spherical, or nearly so, and are made up of one or two nucleoli and finely granular chromatin within the nuclear membrane. (Pl. V, figs. 20-24.) They are a little larger than those of *Rhizopus*. The furrows often cut very close to them, but they give no visible sign of being in any way affected by the cleavage of the cytoplasm in which they lie. (Pl. V, figs. 21-23.) I have never observed a single case of nuclear division in the sporangium of *Phycomyces*.

The very small vacuoles described above that have no stainable contents do not take any part in the cleavage. They remain round throughout the process, even when the furrows from the larger vacuoles cut very close to them. (Pl. V, fig. 22.)

As the vacuoles that take part in the cleavage become angular the content becomes angular also, taking approximately the shape of the vacuoles, so that its surface is parallel to the vacuolar membrane, but seldom in contact with it, there being still the clear nonstainable zone between. (Pl. V, figs. 21 and 22.) As the angles of adjacent vacuoles fuse, the contents are brought in contact and fuse also, thus forming a mass filling up the spaces between the spores. (Pl. V, fig. 21.) It will clearly be seen that this mass is not protoplasm, as it originates as a secretion from the vacuolar membrane deposited inside the vacuole. It is homogeneous at the time the spores are formed, staining bluish-brown in Flemming's triple stain and containing no nuclei or other inclusions. All the cytoplasm and nuclei of the spore-plasm are included within the spores themselves. (Pl. V, fig. 21.)

There appears to be a considerable shrinkage of the protoplasm while the cutting out of the columella and the spore formation are going on, and this is followed by an increased turgidity of the protoplasmic masses, but this is not so marked as in *Rhizopus* and *Pilobolus* and the spores do not become sharply angular. This increase in turgidity of the protoplasmic masses is followed by a very marked enlargement of the small vacuoles, which did not take part in spore formation. They still, however, contain only ordinary cell sap and no stainable contents. The columella wall begins to form while spore cleavage is going on, and continues to thicken until the spores are nearly ripe.

Up to this time the spores are surrounded by only a plasma-membrane, the spore wall not yet having been formed. They now begin to round off and contract, the vacuoles become very much smaller, and the whole spore is thereby much reduced in size and surrounds itself with a wall of considerable thickness. At the time the spore wall is formed the plasma-membranes of the adjacent spores are not in contact, but are separated by the intersporal slime from the vacuolar contents.

The plasma-membranes of the spores, except in the peripheral layer, originate entirely from the vacuolar membranes, without visible change except in form. Only a part of the plasma-membrane of the spores in this layer is made up of the original plasma-membrane of the sporangium. In this respect there is a marked difference between *Phycomyces* and *Rhizopus*.

The spores vary greatly in size and in the number of nuclei. Every spore has at least one nucleus, and some have as many as twelve or perhaps more. As a rule, there are about six or eight. In Pl. V, fig. 25, *a* and *c* show the extreme sizes of the spores and *b* the usual size and shape. Unlike *Rhizopus*, the walls of these spores are smooth.

Occasionally the cleavage is interrupted before it is complete, and walls are built around partially divided masses of protoplasm before they have rounded off sufficiently to obliterate the furrows. This results in peculiar-shaped spores, such as are shown in Pl. V, fig. 26, *e*, and fig. 27.

After the spores have been formed the intersporal slime becomes foamy in appearance. (Pl. V, fig. 24.) If the sporangium be allowed to dry out and is then placed in water, this intersporal substance swells considerably and probably aids in breaking the sporangium wall. This wall is made up of two layers from a stage even younger than that shown in Pl. IV, fig. 15, though the walls of the mycelium and the sporangiophores show only one layer in stained preparations.

Owing to the great shrinkage of the spores in ripening and to the partial collapse of the columella, the sporangium is very much smaller in diameter when mature than at the time the spores are formed.

From the time of the cleavage to the ripening of the spores the sporangiophores elongate very rapidly, often reaching a length of 10 cm. or more. The ripening usually requires only a few hours.

As in *Rhizopus*, the old mycelium is not entirely empty, but contains a very thin layer of protoplasm lying close to the wall, and in this protoplasm are embedded a few nuclei. The columella also in the ripe sporangium contains a loose network of protoplasm with scattered nuclei. The nuclei, while the mycelium is young, have essentially the same structure as those in the spores, except that they often have as many as three nucleoli. As the mycelium grows older, however, they disintegrate like those of *Rhizopus*.

The writer has also studied the cutting out of the columella and the spores in *Pilobolus crystallinus* and *Sporodinia grandis*, but, as his investigations agree with Harper's account (1899), he has simply given a fuller review of his work than he should otherwise have done, and shall treat these two genera in his general discussion.

GENERAL CONSIDERATIONS.

From a consideration of the preceding pages, we find that the processes by which the spores are formed in *Rhizopus* and *Phycomyces* appear very different, and that both these forms differ from *Pilobolus* and *Sporodinia*, which two are different from each other. In other words, of the four genera of the Mucorineæ that have been most carefully studied no two are alike.

In *Phycomyces* the spore-plasm is divided into spores by vacuoles alone.^a The furrows cutting outward from the columella cleft form no exception to this statement, this cleft being simply a fused system of vacuoles. In *Pilobolus* both vacuoles and surface furrows take part in the process. In *Rhizopus* and *Sporodinia* the work is done entirely by surface furrows and furrows from the columella cleft, the vacuoles in the spore plasm playing no part in the process. *Sporodinia*, however, differs from all the other forms in having none of the denser plasm included inside the columella, and from *Pilobolus* and *Rhizopus* in having no surface furrow to assist the vacuoles in cutting out the columella. *Pilobolus* differs also from the other three forms in that the spores in this genus only are cut down to a uninucleated stage, followed by an embryonic development consisting of nuclear and cell division.

There are some respects, however, in which all four genera agree. In all cases the protoplasm is divided progressively, the nuclei during the cleavage are in a resting state, and *all* the protoplasm in the sporangium outside the columella is included within the spore walls, the substance between the spores not being protoplasm but a slimy material excreted by it through osmotic membranes.

Harper (1899) has pointed out that this is not a process of free cell formation in the sense that the cells are cut out entirely within a larger mass of protoplasm, as in the ascus of *Lachnea* and *Erysiphe*, but is an entirely different type of cell division. He uses this as evidence against the homology of the sporangium of the Zygomycetes with the ascus. Juel (1902), however, in a very recent paper on *Taphridium* (a new genus of the Protomycetes) seems to have entirely missed this part of Harper's distinction. He refers to the action of the kinoplasmic rays as being Harper's whole distinguishing characteristic of free cell formation, and considers this insufficient grounds for such a distinction. He

^a By this is meant not that the vacuoles are the sole and active agents of division, but that they are not assisted by surface furrows. See note to page 31.

calls the division of the protoplasm in *Taphridium* free spore formation, though he confesses that he does not understand the stage in which the spores are being cut out, and gives us no conclusive evidence that the substance between the spores is protoplasm.

Timberlake (1902) has described a process of cleavage in the formation of the swarm-spores of *Hydrodictyon urtriculatum* similar to that which I have described. In this alga the protoplasm forms a layer of an even thickness around a central vacuole, and this protoplasm is divided into a single layer of spores by narrow furrows cutting from the central vacuole outward and meeting similar furrows from the surfaces.

The mechanics of this process of division present a very perplexing problem. Sections like those shown in Pl. II, fig. 8, and Pl. III, fig. 10, where cleavage is only partly complete, have an appearance that suggests the effect of cracking on the surface due to drying. If a colloidal sphere were allowed to dry by evaporation from its surface, it would crack and split in a manner much like the sporangia of *Rhizopus*. That such an explanation is not adequate for this cleavage phenomenon is clearly evident from the fact that the furrows are filled with cell sap in living specimens throughout the entire process of division. One can scarcely imagine any body cracking from drying out when the crevices are filled with a watery liquid. In any case such an explanation would not account for cleavage by angles cutting out from vacuoles embedded in the protoplasm.

An explanation that would in a measure account for the angles being pushed out from the vacuoles is that the vacuoles take up water from the surrounding cytoplasm by osmosis through their membranes, which would cause an outward pressure against the latter. If now certain parts of this membrane should become weaker than other parts, these weaker parts would be pushed out by the internal pressure. Such an explanation, however, would not account for the surface furrows, as they are not surrounded on all sides by an osmotic membrane, there being no membrane across the mouth of the furrow at the periphery of the sporangium. (Pl. II, figs. 8 and 9.) If such a membrane be present, it is so thin that it is not visible with the highest powers of the microscope, and hence it is doubtful whether it would be more resistant to outward pressure from within the furrow than the plasma-membrane and cytoplasm at the inner edge of the furrow.

That the plasma-membrane and vacuolar membrane should possess sufficient rigidity to cut into the protoplasm after the fashion of a knife is entirely foreign to our conception of these membranes.

The most probable explanation the writer has found for the mechanics of the cleavage is on the basis of local contractions of the cytoplasm, somewhat comparable to the phenomena exhibited in the naked protoplasm of amoebae and pseudopodia. In Pl. VI, figs. 28-31, the writer

has attempted to demonstrate diagrammatically the way in which such localized contractions would cut up the protoplasm in exactly the same manner actually occurring in these sporangia. The type of cleavage represented in *Pilobolus* has been chosen so that the same diagrams may be used to explain vacuolar and surface-furrow cleavage. For the sake of clearness the diagrams were made much simpler than the actual sporangia, but without changing any essential fact of structure. The lines of force caused by the contraction of the cytoplasm have been represented by arrows—red indicating a locality in maximum contraction; green, a locality that has not yet reached its maximum; and blue, a locality that has passed its maximum. Where there are wide spaces between arrows there is assumed to be little or no contraction. Dotted black lines represent planes where cleavage will take place.

For the cutting out of the columella, let us assume that after the system of vacuoles shown in Pl. VI, fig. 28, is formed, the cytoplasm at such points between but close to the vacuoles, as shown by the red arrows, begins to contract in a direction at right angles to the future columella cleft, the spore-plasm pulling toward the periphery and the columella-plasm toward the center of the sporangium. This would tend to pull the cytoplasm away from the points at the rear ends of the arrows and also to draw the general masses of the spore-plasm and the columella-plasm toward each other. This would cause pressure against the sides of the vacuoles and cause them to flatten out to fill the spaces from which cytoplasm is being withdrawn, as is shown in Pl. VI, fig. 29. At the base of the sporangium where the surface furrow is to cut in, the cytoplasm contracts in a direction approximately radial to the curve in which the furrow is to cut. This pulling causes a rift beginning at the surface of the sporangium, and the viscid plasma-membrane, ever adhering to the surface of the cytoplasm, folds in to line this rift. As the furrow cuts inward, the points of greatest contraction move inward also (Pl. VI, fig. 29), keeping always close in front of the furrow until the latter fuses with the lowest vacuoles in the system.

The principle involved in the cleavage of the spore-plasm is essentially the same. At the points indicated by red arrows in Pl. VI, fig. 30, viz, on the periphery, on the columella cleft, and on the vacuoles, the cytoplasm begins to contract in a direction approximately toward the centers of the masses of protoplasm that are to become the spores. This pulling away of the protoplasm causes rifts or furrows running into the spore-plasm from the periphery, the columella cleft, and the vacuoles, as shown in Pl. VI, fig. 31. The width of the furrows depends on the continuation of the contraction after the furrows have progressed beyond the points of contraction, i. e., on the amount of contraction that takes place at the points marked in the diagrams

by green-colored arrows. If the pulling at the sides of the furrows continues, as in *Pilobolus*, the furrows are wide, but if it soon ceases, as in *Synchitrium*, they are narrow.

It is not improbable that in the last stages of cleavage, where the spores are connected by only a slender neck, constriction like that which cuts off conidia may play a part in finishing the process.

There is little evidence that the nuclei directly influence the contraction. The direction of the contraction seems to be in general toward the center of the protoplasmic masses that are to be the spores, without regard to the distribution of the nuclei. The nuclei do, however, seem to determine to some extent just what protoplasm shall constitute each individual spore; otherwise we might have spores formed of enucleated pieces of protoplasm, and none such has ever been observed in these forms.

Viewing the cleavage from the basis of localized contraction of the cytoplasm, we do not find such radical differences in the processes involved in *Pilobolus*, *Sporodinia*, *Rhizopus*, and *Phycomyces* as appeared at first sight. In *Pilobolus* and *Phycomyces* there are large vacuoles in the spore-plasm, in the vicinity of which cytoplasmic contractions take place in such a way as to cause angles to cut outward from the vacuoles, while in *Sporodinia* and *Rhizopus* such is not the case. On the other hand, there are no cytoplasmic contractions on the periphery of the sporangium in *Phycomyces* as in the other three genera. Otherwise these four genera exhibit no essential differences in the manner of formation of the columella and spores. The difference is simply in the location of the cytoplasmic contractions.

The explanation offered for the mechanics of the cleavage in the sporangia of the Mucorineæ seems equally applicable to other cases of surface cleavage, e. g., *Synchitrium*, *Fuligo*, and some animal eggs. To illustrate this extended application of the theory the writer has made diagrams of *Synchitrium*, *Fuligo*, and the egg of the squid, indicating, by means of arrows, as in Pl. VI, figs. 28-31, the location, direction, and duration of the cytoplasmic contractions that would produce such furrows as have been observed in these forms. Pl. VI, figs. 32 and 33, are based on Harper's (1899 and 1900) figures, and Pl. VI, fig. 34, on Watasé's (1890) figure. If this view of the mechanics of cleavage be the correct one, we must regard the vacuoles as passive rather than active agents in cutting the protoplasm.^a They have, however, a very definite and important mission to perform. In all four genera under discussion they form the greater part of the plasma-membrane for the columella and for the surface of the spore-plasm next to the columella, and in *Pilobolus* and *Phycomyces* they form the greater part of the plasma-membrane for the spores. As I have already stated, this is done by the vacuolar membrane becoming directly a

^a See note at the bottom of page 28.

part of the plasma-membrane without any visible change except in form. The protoplasmic surface that abutted against the vacuole is the same that is later in contact with the cell sap in the clefts. The boundary of the vacuole has become directly the boundary of a part of the cleft. We have good reason, therefore, to believe that the vacuolar membrane is identical with, or at least very similar to, the plasma-membrane, and may serve the same purpose if opportunity is offered. This homology is further substantiated by the fact that the columella wall is laid down in the dome-shaped vacuolar cleft by the plasma-membranes, formed for the most part by the vacuolar membranes, and, in the case of *Phycomyces* and *Pilobolus*, the walls of most of the spores are formed by what was once a number of vacuolar membranes. If, with Strasburger (1898), we regard the plasma-membrane as kinoplasmic, we find here very strong reasons for believing that the vacuolar membrane is of a kinoplasmic nature also.

The vacuoles are, then, openings in the protoplasmic mass, less resistant to the contraction of the cytoplasm, and from which clefts may originate. In the higher plants and in the ascus of the Ascomycetes we have the new plasma-membrane of the daughter cells formed by the kinoplasmic fibers. In most animal cells and in many of the algæ, as *Cladophora*, and in the formation of conidia in fungi, the new plasma-membrane originates from the old by following the constriction furrow from the surface inward. In *Phycomyces* there are neither spindle fibers nor surface furrows present during spore formation, and the kinoplasm which forms the plasma-membranes for the spores seems to be located entirely in the vacuolar membrane.

The behavior of the vacuoles in the sporangia of *Pilobolus*, *Sporodinia*, *Rhizopus*, and *Phycomyces* is of considerable interest in its bearing on the question of whether or not the vacuole can be considered as a permanent organ of the cell. Though, as already suggested, the vacuoles are probably not active agents in the division of the protoplasm, yet there can be no doubt that they do have a part to play in the process by offering places of slight resistance to the contractions of the cytoplasm, and by supplying material for the formation of new plasma-membranes around the spores and the columella. In the cutting out of the columella it is evident that the vacuoles are arranged in their definite dome-shaped system for the distinct purpose of being where they can best do their part in the process. In *Phycomyces* the early formation of the stainable substance in some vacuoles, while others remain empty, and the fact that the former go to form plasma-membranes for the spores and the columella, while the latter do not, indicate that certain vacuoles are predestined from a very young condition of the sporangium to take part in columella and spore formation.

The idea that the vacuolar membrane has special properties not possessed by the general body of the cytoplasm is by no means a new one. De Vries (1885) has shown, by treating living cells with plasmolyzing agents containing coloring matter, that the vacuole wall is an osmotic membrane like the *hautschicht*. He has also been able to isolate the vacuoles from the cytoplasm without breaking them, showing the wall to have some strength and elasticity, and that it retains its identity even when not surrounded by a viscid cytoplasm. The vacuoles of *Spirogyra* were often seen to divide by constriction when treated with a saltpeter solution. By long immersion in a saltpeter solution followed by eosin the vacuole wall was hardened, so that it would be broken by pressure without collapsing. De Vries concludes that there is a very strong similarity between vacuole wall and *hautschicht*.

Went (1888) holds that all living plant cells, with the possible exception of bacteria, Cyanophyceae, and spermatozooids, contain vacuoles, which by division furnish all the vacuoles for the succeeding generations of cells. In *Aspergillus oryzae* he saw both division and fusion of vacuoles. In a cell of *Dematium pullulans* he observed nine vacuoles fuse into two large ones. These then fused to form one; but before the constriction left at the point of fusion had disappeared another constriction had begun to form in another part of the same vacuole, which increased in depth until it had cut the vacuole in two again. Went expresses his belief that the wall of the vacuole plays an active part in this division. In *Cladosporium herbarum* and in the hairs on the epidermis of *Cucurbita pepo* he found that the vacuoles divide just before cell division.

Went concludes that the vacuole wall is an organ of the protoplasm ranking with the nucleus and the chromatophores, originating by the division of a previously existing vacuole, and never forming *de novo* in the protoplasm.

Bokorny (1893) treated living cells with a weak caffeine solution and found that the vacuole wall was not killed by it, but that it contracted without losing its rounded outline, precisely as De Vries describes for vacuoles when the cell is treated with a 10 per cent saltpeter solution. Bokorny points out that, as a dilute caffeine solution has but very weak plasmolyzing power, the phenomenon in this case is one of irritability, the caffeine solution being the stimulus and the vacuole wall being the receptive part of the cell. A caffeine solution as weak as 0.01 per cent will cause the reaction.

The work of these authors offers very strong evidence that the vacuolar membrane is at least a differentiated and specialized portion of the protoplasm, differing molecularly from ordinary cytoplasm, and having many properties in common with the plasma-membrane.

Pfeffer (1890) confirms the conclusions of De Vries and Went that the vacuole wall is an osmotic membrane very much like the hautschicht, and that it reproduces itself by division. He is able to form new vacuoles in plasmodia, however, by introducing very small particles of asparagin, and finds these to agree in all essential particulars with normally produced vacuoles. He also holds that by extensive vacuolization nearly all of the cytoplasm may be changed to "plasma-haut" (vacuole wall and hautschicht). Pfeffer, seems, however, inclined to regard both the vacuole wall and the hautschicht as the result of surface tension, and a precipitation of the surface of the cytoplasm by contact with water.

Bütschli (1892) also refuses to accept the view that the vacuole is bounded by a definite, permanent wall, and would, with Pfeffer, refer it to surface tension between the watery liquid of the vacuole and the viscid, semiliquid protoplasm; or, at most, he regards this boundary as only a precipitation membrane formed by the action of the vacuolar contents on the adjacent protoplasm.

In the part played by the vacuoles in the formation of the spores in the Mucorineæ we have additional evidence that the vacuolar membrane is a more definite structure than Bütschli regards it. The vacuolar membrane so clearly is able at times to perform the functions of the plasma-membrane that the structure and composition of the two seem identical.

The exact composition of the stainable substance in the vacuoles of *Phycomyces* is not easy to determine. In living sporangia crushed under a cover-glass it is easy to see the vacuoles more or less isolated from the cytoplasm, but no contents can be seen at any stage of development. Neither can the intersporal substance be seen in sporangia where cleavage is only partially complete. In older sporangia, however, this is clearly visible. This would suggest that this substance is in solution or very transparent in living sporangia and is precipitated in the process of dehydrating or clearing—probably by the alcohol. It appears in sections of old sporangia even before staining, no matter what fixing fluid is used. It is not readily soluble in water, as may be seen from the fact that it is visible in sections that have been soaked several hours in water.

In seeds, Wakker (1888) found that the aleurone grains inside the vacuoles begin as very minute, dense bodies, much smaller than the vacuoles themselves, afterwards increasing in size till the vacuoles are nearly or quite filled by them. As I have already pointed out, however, the contents of the vacuoles of *Phycomyces* are at the moment they first become visible quite as large in proportion to the size of the vacuole as when they become older. The substance seems to be evenly distributed in the cell sap of the vacuole, simply increasing in density as the sporangium grows older. There can be little doubt that it is a

secretion of the cytoplasm through the vacuolar membrane, and the fact that the substance is secreted only in those vacuoles which are to take part in the cleavage seems to indicate a difference, in function at least, between the membranes of the two kinds of vacuoles.

The clear zone between the body inside the vacuole and the vacuolar membrane seems to be due to the contraction of the substance in dehydration.

The fact that this substance takes so readily the shape of the vacuole or the furrow that contains it would show that in the living state it is not solid, but very plastic, if not in actual solution.

Stevens (1899) describes a gelatinous, stainable substance in the vacuoles of the oogonium of *Albugo bliti*, and seems to regard it as being used to form the walls of the oospore. He says of it: "It appears to be transferred directly from the vacuoles to the exterior of the protoplasm, there to be changed to true cellulose." Whether or not this substance is the same as that in *Phycomyces* I can not be certain. Stevens's description agrees very well with my own in that the substance takes the stain only slightly when first formed, and stronger in later stages. In *Albugo*, however, it leaves the vacuoles and goes to form cellulose walls, while in *Phycomyces* it never disappears, but forms the inter-spore substance in the clefts made by the vacuoles and apparently plays no part in the formation of walls. Stevens describes this substance as occurring in figs. 91, 92, 93, and 94 of his Pl. XV, but I have been unable to find any representation of it in the places referred to. Neither does he describe the method by which it is transferred to the periphery of the oospore.

Trow (1901) also has figured a similar content in the vacuoles of *Pythium ultimum*, but does not describe it so as to give any idea of its true nature.

A problem that has been most perplexing to me is how the protoplasm in the sporangium comes to be differentiated into a very dense layer at the periphery containing many nuclei, and a very loose structure in the interior with few nuclei. The purpose of such a differentiation is very evident, viz: That as much of the protoplasm as possible may be included within the spores, but just what propels the protoplasm up the sporangiophore and out toward the periphery of the sporangium is not so easy to determine. Arthur's (1897) explanation that it is due to evaporation of water from the surface, combined with absorption of moisture from the substratum, seems entirely inadequate. If this were the cause, we should expect to find the layer of denser protoplasm at the base of the sporangium as well as on the sides and top, as we have no evidence that evaporation does not go on from the part of the sporangium just around the sporangiophore as well as from the rest of the surface. Furthermore, from Arthur's explanation we should expect a gradual transition between the denser

and the less dense protoplasms, but, as has been already pointed out, the transition is quite sudden. Still further, the layer of dense protoplasm is not of the same absolute thickness for all sporangia, nor does it bear a constant relation to the size of the sporangium. The writer has been rather inclined to regard the thickness of this layer as dependent on the amount of available protoplasm, though by no means certain on this point. Arthur's conclusions bear more specifically on the streaming in the hyphæ than in the sporangium, and yet he gives us no intimation that he wishes to separate the two processes and refer them to different causes.

In the formation of the oosphere in some of the Peronosporæ we have, according to Wager (1896), Stevens (1899), and others, a differentiation of the protoplasm into ooplasm and periplasm, but this differentiation is not characterized by such a marked difference in the density of the two protoplasms as in the Mucorinæ. The wall about the oosphere is described as forming on the boundary between two protoplasms. The question as to just how the protoplasm is divided and the wall formed has been pretty carefully avoided by all these authors. Stevens states that there is a thin film formed between the two protoplasms, and that this film seems to develop into the wall of the oospore, but his account of the process is very incomplete. Trow (1901) figures a stage in *Pythium ultimum*, in which the oosphere is only partially cut out, but, unfortunately, he does not describe it sufficiently to give us a clear conception of the real nature of the process.

The fact that the columella cleft forms just inside the denser plasm, rather than between it and the looser plasm, accords well with the idea that the cleft is formed by cytoplasmic contractions. The layer of denser plasm inside the columella cleft seems to be for the specific purpose of aiding in the cleavage by its contraction, a function that the looser plasm is probably unable to perform.

SUMMARY.

The essential processes in the formation of the spores in the sporangia of *Rhizopus* and *Phycomyces* may be summarized as follows:

1. Streaming of the cytoplasm nuclei and vacuoles up the sporangio-phore and out toward the periphery, forming a dense layer next the sporangium wall and a less dense region in the interior, both containing nuclei.
2. Formation of a layer of comparatively large, round vacuoles in the denser plasm parallel to its inner surface.
3. Extension of these vacuoles by flattening so that they fuse to form a curved cleft in the denser plasm; and, in the case of *Rhizopus*, the cutting upward of a circular surface furrow from the base of the sporangium to meet the cleft formed by these vacuoles, thus cleaving out the columella.

4. Division of the spore-plasm into spores; in *Rhizopus*, by furrows pushing progressively inward from the surface and outward from the columella cleft, both systems branching, curving, and intersecting to form multinucleated bits of protoplasm, surrounded only by plasma-membranes and separated by spaces filled with cell sap only; in *Phycomyces*, by angles forming in certain vacuoles containing a stainable substance and continuing outward into the spore-plasm as furrows, aided by other furrows from the columella cleft and dividing the protoplasm into bits homologous with and similar to those in *Rhizopus*, and separated by furrows partly filled with the contents of the vacuoles that assist in the cleavage.

5. Formation of walls about the spores and columella, and, in the case of *Rhizopus*, the secretion of an intersporal slime.

6. Partial disintegration of the nuclei in the columella.

INDEX TO LITERATURE.

- ARTHUR, J. C. (1897): The Movement of Protoplasm in Cœnocytic Hyphæ. *Ann. of Bot.* 11, 1897, p. 491.
- BACHMANN, H. (1899): *Mortierella van Tieghemi*. Beitrag zur Physiologie der Pilze. *Jahrb. für wiss. Bot.* xxxiv, 1899, p. 279.
- BOKORNY, TH. (1893): Die Vakuolenwand der Pflanzenzellen. *Biol. Centr.* 13, 1893, p. 271.
- BÜSGEN, M. (1882): Die Entwicklung der Phycomycetensporangien. *Jahrb. für wiss. Bot.* XIII, 1882, p. 253.
- BÜTSCHLI, O. (1892): Untersuchungen über mikroskopische Schäume und das Protoplasma, 1892, p. 145 et al.
- CORDA, A. C. J. (1838): *Icones Fungorum*, II, 1838, p. 19.
- DE VRIES, H. (1885): Plasmolytische Studien über die Wand der Vacuolen. *Jahrb. für wiss. Bot.*, XVI, 1885, p. 465.
- FISCHER, A. (1892): Phycomycetes. Rabenhorst's Kryptogamen-flora. Band I, Abt. IV, 1892, p. 161 et al.
- HARPER, R. A. (1899): Cell-Division in Sporangia and Asci. *Ann. of Bot.*, 13, 1899, p. 467.
- (1900): Cell and Nuclear Division in *Fuligo varians*. *Bot. Gaz.*, 30, 1900, p. 217.
- JUEL, H. O. (1902): Taphridium. Bihang till K. Sv. Vet. Akad. Handl, 1902.
- LÉGER, M. (1896): Recherches histol. sur la structure des Mucorinées, 1896.
- PFEFFER, W. (1890): Zur Kenntniss der Plasmahaut und der Vacuolen. *Abh. d. k. sächs. Ges. d. Wiss.*, XVI, 1890, p. 187.
- STEVENS, F. L. (1899): The Compound Oosphere of *Albugo Bliti*. *Bot. Gaz.*, 28, 1899, p. 149.
- STRASBURGER, E. (1880): Zellbildung und Zelltheilung, 3d ed., 1880.
- (1898): Die pflanzlichen Zellhäute. *Jahrb. für wiss. Bot.*, XXXI, 1898, p. 511.
- TROW, A. H. (1901): Observations on the Biology and Cytology of *Pythium ultimum*. *Ann. of Bot.*, 15, 1901, p. 269.
- THAXTER, R. (1897): New or Peculiar Zygomycetes. 2. Syncephalastrum and Syncephalis. *Bot. Gaz.*, 24, 1897, p. 1.
- TIMBERLAKE, H. G. (1902): Development and Structure of the Swarm-spores of *Hydrodictyon*. *Trans. Wis. Acad. Sci.*, XIII, 1902, p. 486.
- VAN TIEGHEM, Ph., et G. LE MONNIER (1873): Recherches sur les Mucorinées. *Ann. d. sc. nat.*, 5^e sér., Bot., 17, 1873, p. 261.
- VAN TIEGHEM, Ph. (1875): Nouvelles Recherches sur les Mucorinées. *Ann. d. sc. nat.*, 6^e sér., Bot., 1, 1875, p. 5.
- (1876): Troisième Mémoire sur les Mucorinées. *Ann. d. sc. nat.*, 6^e sér., Bot., 4, 1876, p. 312.
- WAGER, H. (1896): On the Structure and Reproduction of *Cystopus candidus*. *Ann. of Bot.*, 10, 1896, p. 295.
- WAKKER, J. H. (1888): Studien über die Inhaltskörper der Pflanzenzelle. *Jahrb. für wiss. Bot.*, XIX, 1888, p. 423.
- WATASE, S. (1890): Studies on Cephalopods: I. Cleavage of the Ovum. *Jour. of Morph.*, IV, 1890, p. 247.
- WENT, F. A. F. C. (1888): Der Vermehrung der normalen Vacuolen durch Theilung. *Jahrb. für wiss. Bot.*, XIX, 1888, p. 295.

DESCRIPTION OF PLATES.

[All the figures were drawn with the aid of a Leitz or a Zeiss camera lucida, with objectives and oculars, as follows: Fig. 1, Leitz No. 1 objective, No. 0 ocular; figs. 5, 6, 7, 8, 10, and 12, Leitz $\frac{1}{15}$ oil-immersion objective, No. 0 ocular; fig. 14, Leitz $\frac{1}{15}$ oil-immersion objective, No. 3 ocular; figs. 15, 17, 18, and 19, Zeiss 2 mm. 1.30 aperture, oil-immersion objective, No. 1 Huyghenian ocular; figs. 2, 3, 21, 24, 26, and 27, Zeiss 2 mm. 1.30 aperture, oil-immersion objective, No. 6 compensating ocular; figs. 22, 23, and 25, Zeiss 2 mm. 1.30 aperture, oil-immersion objective, No. 12 compensating ocular; figs. 4, 9, 11, 13, 16, and 20, Zeiss 2 mm. 1.30 aperture, oil-immersion objective, No. 18 compensating ocular.]

PLATE I. *Rhizopus nigricans*. Fig. 1.—Group of sporangiophores bearing sporangia, showing how they grow out from the stolon. $\times 12$. Fig. 2.—Longitudinal section of young stolon, showing distribution of cytoplasm and nuclei. $\times 750$. Fig. 3.—Same, except that the stolon is much older; wall very thick, and nuclei disintegrating. $\times 750$. Fig. 4.—Disintegrating nuclei from stolon shown in fig. 3. $\times 2,250$. Fig. 5.—Young sporangium, showing cytoplasm and nuclei streaming up the sporangiophore into the sporangium and out toward the periphery. There are a few crystalloids in the center. $\times 520$. Fig. 6.—Sporangium that has attained nearly its full size. The differentiation between the looser and the denser plasms is sharply marked, except at a few places. Just inside the denser plasm is a clear zone of protoplasm that does not take the orange stain, and through this run strands of orange-staining cytoplasm bearing nuclei. $\times 520$.

II. *Rhizopus nigricans*. Fig. 7.—Full-sized sporangium, showing layer of vacuoles nearly formed in the denser plasm. The two layers of the wall are here shown. $\times 520$. Fig. 8.—Section cut through sporangium a little to one side of the sporangiophore. The columella cleft is being formed by fusion of the vacuoles shown in fig. 7, and by a surface furrow. The spores are also being cut out by progressive surface furrows. $\times 520$. Fig. 9.—A small part of the same sporangium as shown in fig. 8, drawn from another section, showing in detail very early cleavage furrows, and structure, size, and distribution of nuclei and vacuoles. $\times 2,250$.

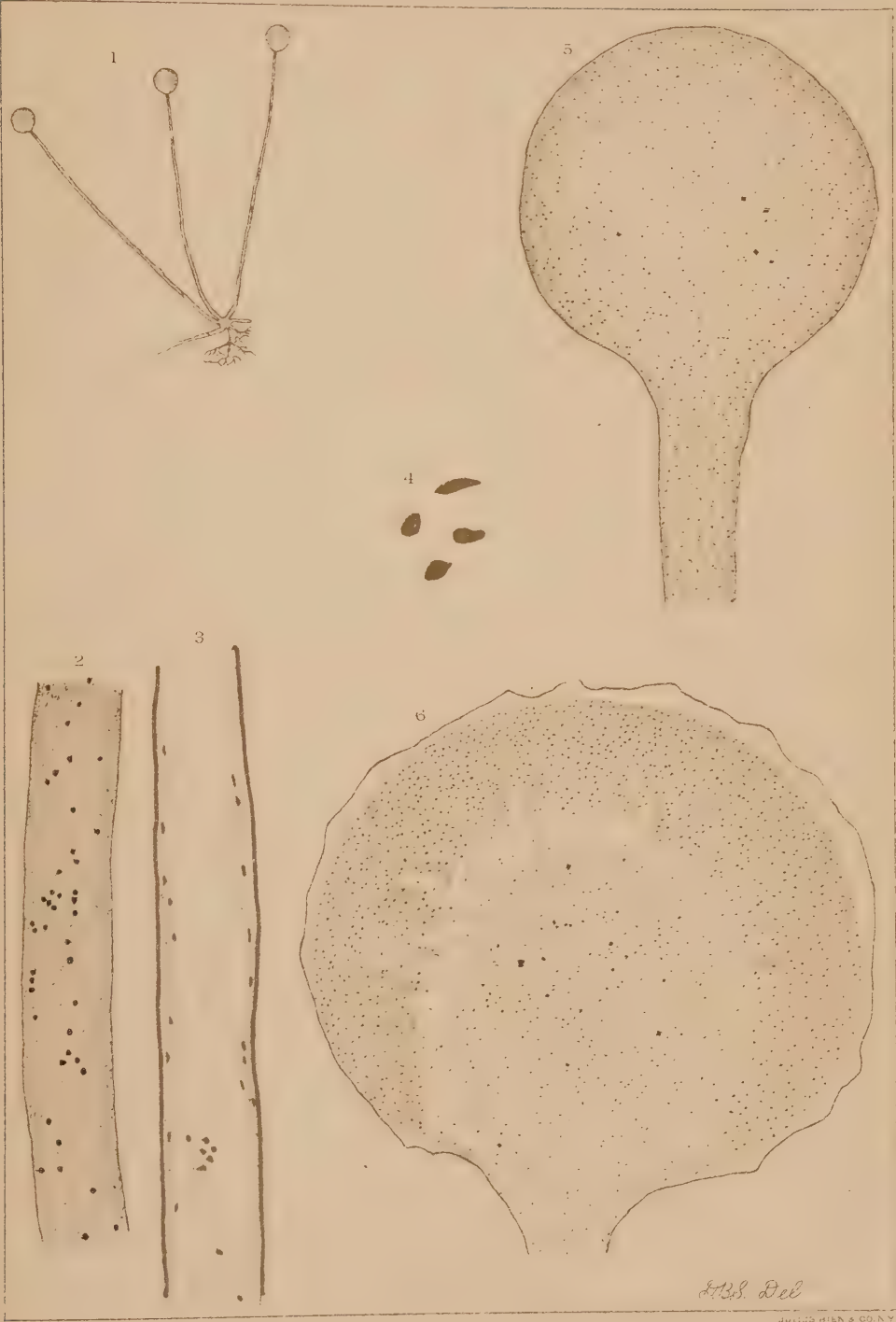
III. *Rhizopus nigricans*. Fig. 10.—Cleavage much farther advanced than in figs. 8 and 9. Furrows cutting outward from the columella cleft. Section not cut through sporangiophore. $\times 520$. Fig. 11.—Nuclei from columella of same; *a*, very close to columella cleft; *b*, *c*, and *d*, nearer the center; *a* has a normal structure, while *b*, *c*, and *d* show stages in disintegration. $\times 2,250$. Fig. 12.—Sporangium in which the spores are completely formed, rounded up, and surrounded by thin walls. The columella wall is also formed. $\times 520$. Fig. 13.—Nuclei from columella of same; *a* lies near columella wall and still retains its normal structure; *b* lies near it but is beginning to disintegrate; *c* and *d* lie near the center and are reduced to homogeneous angular masses. $\times 2,250$. Fig. 14.—Ripe spores in their living condition, showing variations in size and ridges on walls. $\times 950$.

IV. *Phycomyces nitens*. Fig. 15.—Young sporangium, showing cytoplasm nuclei and vacuoles streaming up the sporangiophore and out toward the periphery of the sporangium. Vacuoles in the denser protoplasm have a visible content. $\times 550$. Fig. 16.—Small part of young sporangium very highly magnified, showing early stage in the formation of the visible

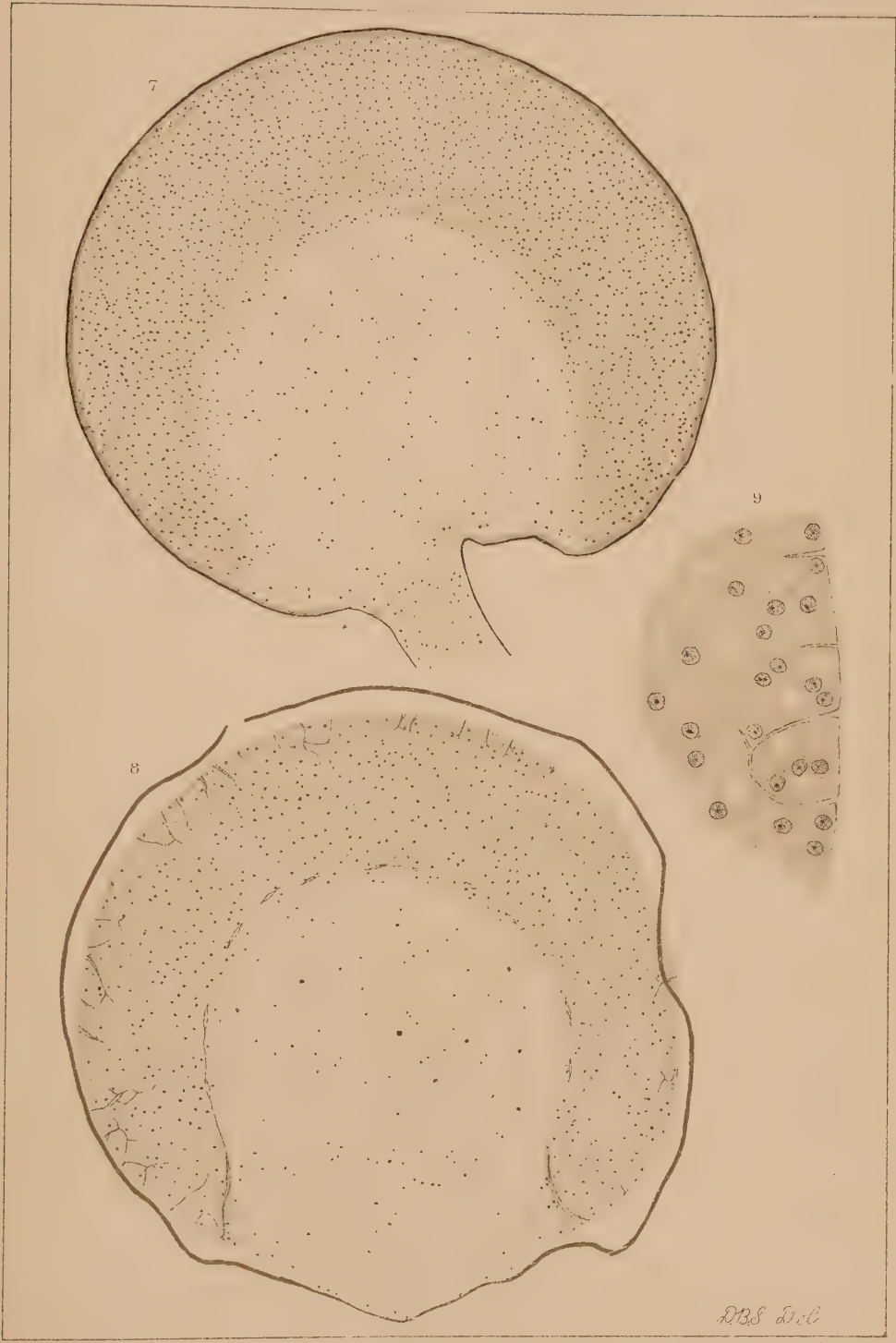
content of the vacuole; also two much smaller vacuoles with no such contents; nuclei in resting condition. $\times 2,250$. Fig. 17.—Portion of cross section of sporangium at a somewhat later stage than fig. 15, showing distribution of protoplasm into an outer dense layer, an interior region of very loose protoplasm containing empty vacuoles and no nuclei, and between these two a layer of intermediate density. $\times 550$. Fig. 18.—Part of longitudinal section of sporangium, showing layer of vacuoles forming in denser protoplasm where the columella is to be cut out. $\times 550$.

PLATE V. *Phycomyces nitens*. Fig. 19.—Layer of vacuoles in the denser plasm, flattening out toward each other to form the columella cleft by their fusion. The contents flatten out also, taking the shape of the vacuoles. $\times 550$. Fig. 20.—Small part of section very highly magnified, showing three vacuoles in contact, separated only by their membranes; also three very small empty vacuoles and six nuclei in resting condition. $\times 2,250$. Fig. 21.—Spore plasm being cut up into spores by vacuoles becoming angular, and the angles cutting through the protoplasm as furrows; cytoplasm in front of furrows undifferentiated; nuclei in a resting condition. The contents of the vacuoles extend out into the furrows and fuse as the furrows fuse, to form the intersporal substance. $\times 750$. Fig. 22.—Furrows cutting outward into the spore plasm from the columella cleft; cytoplasm in front of furrows undifferentiated. $\times 1,500$. Fig. 23.—Furrows from the vacuoles cutting out to the plasma-membrane at the periphery of the sporangium. $\times 1,500$. Fig. 24.—Nearly ripe spores containing resting nuclei and empty vacuoles, and embedded in intersporal slime. $\times 750$. Fig. 25.—Living, ripe spores; walls smooth; *a*, very large; *b*, average size; *c*, very small. $\times 1,500$. Fig. 26.—Very large peculiar-shaped spores; *e*, probably due to arrested cleavage. $\times 750$. Fig. 27.—Very large, irregular-shaped spore due to arrested cleavage. $\times 750$.

VI. *Pilobolus crystallinus*. (Diagrammatic and much simplified.) Fig. 28.—One-half of longitudinal section of sporangium just before the cutting out of the columella. The arrows indicate lines of contraction of the cytoplasm to form the columella cleft. Green arrows indicate points where the contraction is just beginning and red arrows points where the contraction is at its maximum strength; dotted black lines represent planes where cleavage is to take place. Fig. 29.—Same, but somewhat older stage; vacuoles flattened to fill the spaces where the cytoplasm has been pulled away; also surface furrow at the base of the sporangium. Blue arrows indicate points where contraction has passed its maximum strength. Fig. 30.—Columella cleft completed, spore formation just ready to begin. Fig. 31.—Vacuoles in the spore-plasm becoming angular, and furrows cutting inward from the periphery and outward from the columella cleft, due to the cytoplasm pulling away at these points. Fig. 32.—*Synchytrium decipiens*. (After Harper.) Two cleavage furrows cutting into the sporangium. These are slightly open at the inner extremity where the cytoplasm is contracting, but closed nearer the periphery of the sporangium where contraction has ceased. Fig. 33.—*Fuligo varians*. (After Harper.) Two furrows cutting into the spore plasm; furrows slightly open throughout their entire extent. Fig. 34.—Squid. (After Watasé.) Surface view of egg, showing cleavage furrows cutting into the cytoplasm between the nuclei; furrows very narrow at the extremities.



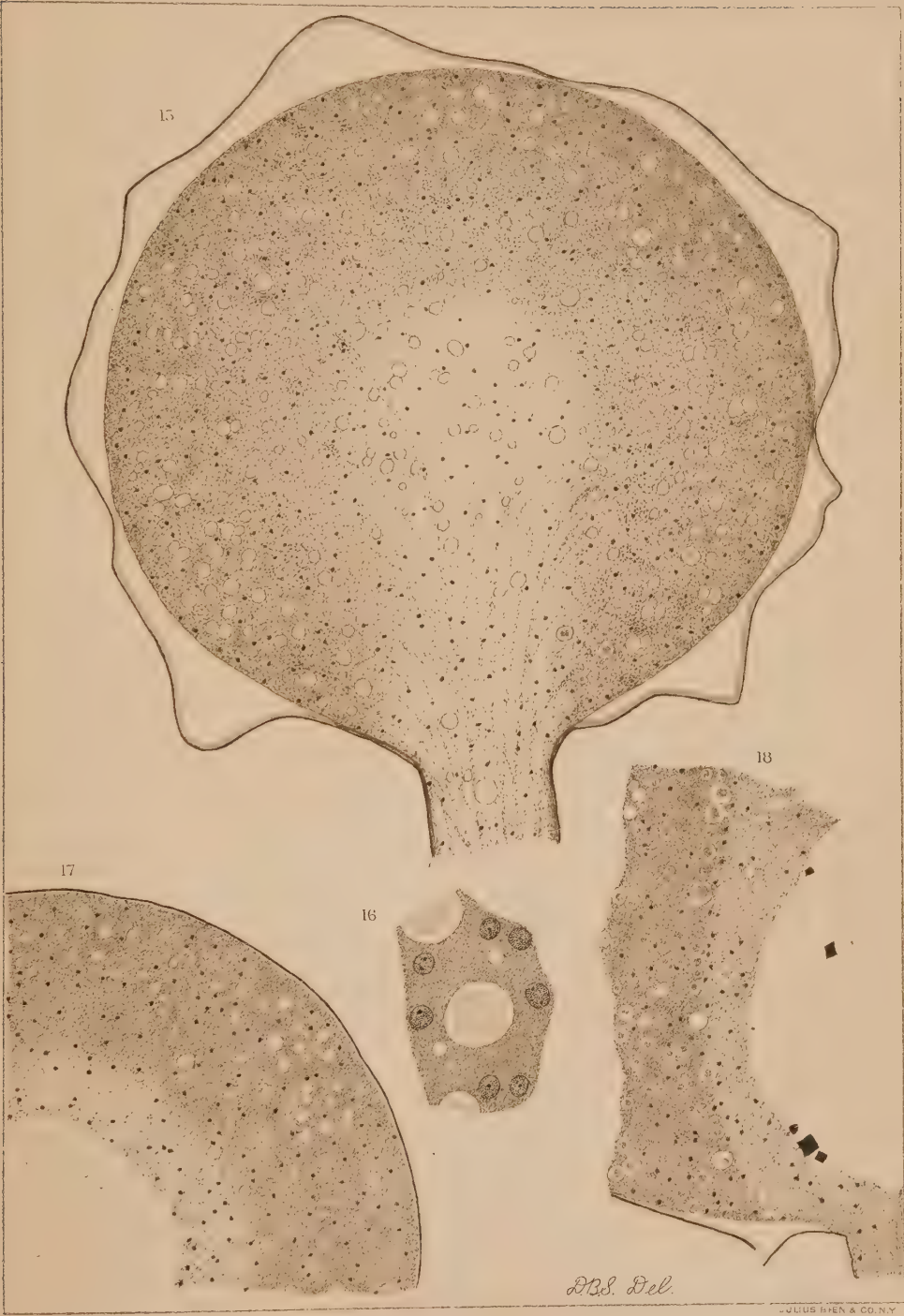
FORMATION OF THE SPORES IN RHIZOPUS NIGRICANS AND PHYCOMYCES NITENS



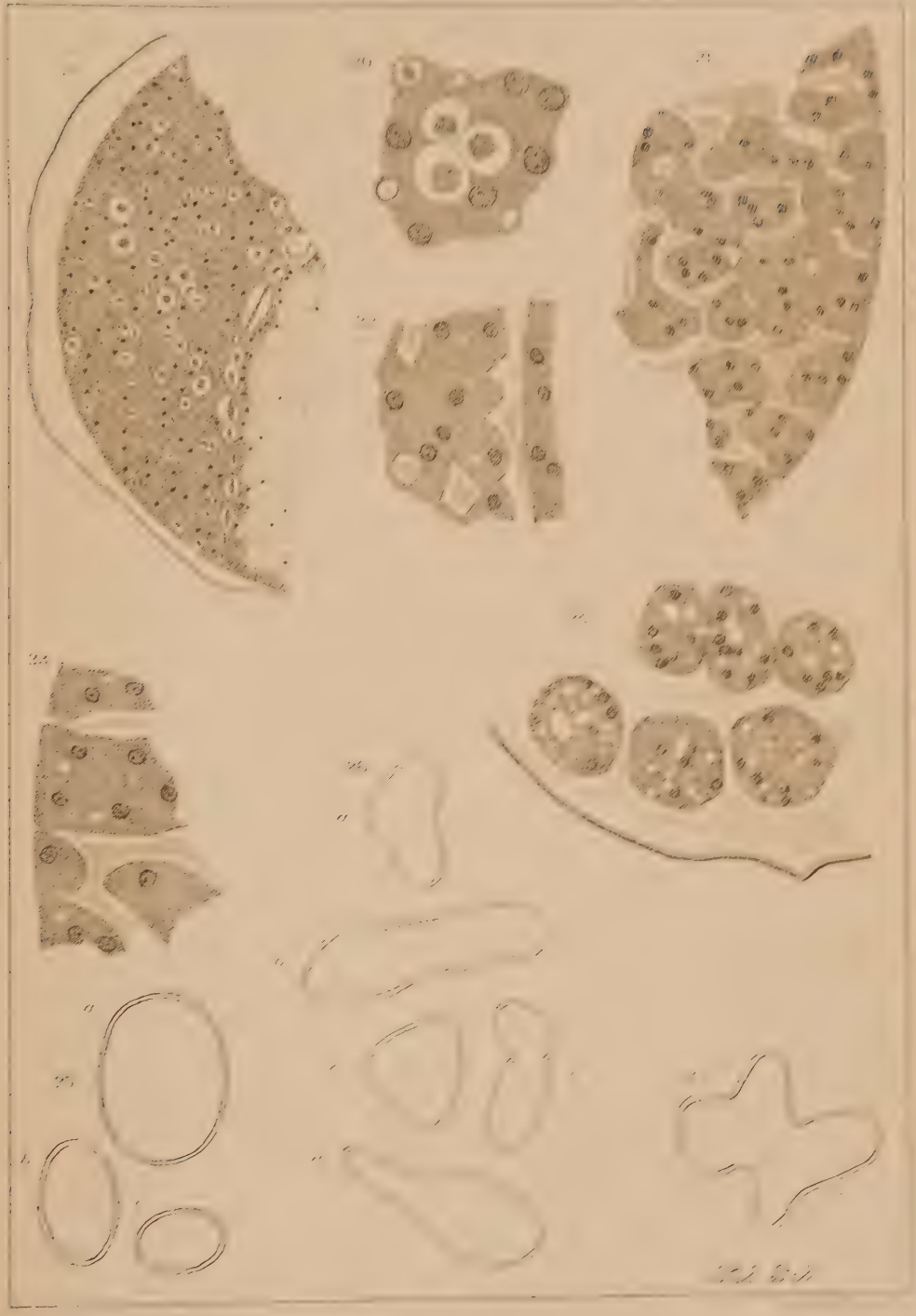
FORMATION OF THE SPORES IN RHIZOPUS NIGRICANS AND PHYCOMYCES NITENS



FORMATION OF THE SPORES IN RHIZOPUS NIGRICANS AND PHYCOMYCES NITENS



FORMATION OF THE SPORES IN RHIZOPUS NIGRICANS AND PHYCOMYCES NITENS



FORMATION OF THE SPOREPHYTE IN THE ZEPHYRUS MOSSES AND PLEUROZETES MOSSES.



FORMATION OF THE SPORES IN RHIZOPUS NIGRICANS AND PHYCOMYCES NITENS

[Continued from page 2 of cover.]

No. 22. Injurious Effects of Premature Pollination; with General Notes on Artificial Pollination and the Setting of Fruit without Pollination. 1902. Price, 10 cents.

No. 23. Berseem: The Great Forage and Soiling Crop of the Nile Valley. 1902. Price, 15 cents.

No. 24. The Manufacture and Preservation of Unfermented Grape Must. 1902. Price, 10 cents.

No. 25. [To consist of miscellaneous papers, of which only one has so far been published, as follows:] The Seeds of Rescue Grass and Chess. 1902.

No. 26. Spanish Almonds and Their Introduction into America. 1902. Price, 15 cents.

No. 27. Letters on Agriculture in the West Indies, Spain, and the Orient. [In press.]

No. 28. The Mango in Porto Rico. 1903. Price, 15 cents.

No. 29. The Effect of Black Rot on Turnips: A Series of Photomicrographs, Accompanied by an Explanatory Text. 1903. Price, 15 cents.

No. 30. Budding the Pecan. 1902. Price, 10 cents.

No. 31. Cultivated Forage Crops of the Northwestern States. 1902. Price, 10 cents.

No. 32. A Disease of the White Ash Caused by Polyporus Fraxinophilus. 1903. Price, 10 cents.

No. 33. North American Species of Leptochloa. 1903. Price, 15 cents.

No. 34. Silkworm Food Plants: Cultivation and Propagation. 1903. Price, 15 cents.

No. 35. Recent Foreign Explorations, as Bearing on the Agricultural Development of the Southern States. 1903. Price, 15 cents.

No. 36. The "Bluing" and the "Red Rot" of the Western Yellow Pine, with Special Reference to the Black Hills Forest Reserve. 1903. Price, 30 cents.

